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WILLIAM MELVILLE WARE

CONTRIBUTORS and readers will share the shock caused to his friends by the sudden death of Dr. Ware on the 15th February, 1955. Ware was only 62 years of age and it seemed that he had before him many years of active life.

Since 1948, when he became Assistant Editor of the *Journal of Horticultural Science*, most of the manuscripts submitted for publication have passed through his hands and there must be few contributors whose efforts have not gained in lucidity and polish from his meticulous and able editing. Ware's flair for the literary presentation of scientific papers was doubtless developed by a youthful grounding in the Classics at Dover College and by the study of modern languages at an academy at Le Havre. The editing of scientific papers was, however, an occupation which he had adopted in his retirement, following a distinguished career of research at Wye College.

Ware entered Wye College in 1911 and, while still a student, showed his interest in investigation by assisting Dr. J. Vargas Eyre in flax selection during summer vacations. In 1914 he joined the Wye College Contingent of The Buffs and later saw active service in France and Belgium with the Royal Engineers. On demobilization he joined the Botany Department of the College as lecturer, but was soon again devoting his leisure to research, this time on problems relating to clovers. By 1923 he had published a number of papers, including two on diseases of Red Clover.

The high quality of Ware's work attracted attention and so, after completing four years in the Botany Department, he was invited by Professor E. S. Salmon to transfer to the Mycology Department (Research and Advisory) to fill the vacancy left by Dr. H. Wormald who had taken up the appointment of Mycologist at East Malling Research Station. Ware began his new duties as Assistant Mycologist in January, 1924, having first attended a short refresher course at the Imperial College of Science.

The next 22 years was a period of solid achievement and a glance at any publication concerned with the plant diseases of Great Britain will show the magnitude of his contributions. About a hundred original papers were published under his name, either alone or jointly with colleagues and they relate to a wide range of horticultural and agricultural crops. But his main work was done on fruit, on hops and on the cultivated mushroom ; among these his outstanding researches related to Apple Scab (*Venturia inaequalis* (Cooke) Wint.) and Hop Downy Mildew (*Pseudoperonospora humili* (Miyabe and Tak.) G. W. Wilson). With both he made major contributions on the biology of the organisms and on the control of the diseases which they caused.

His attention was drawn to disorders of mushrooms by failures in local houses. It was while investigating these that he realized the want of literature on the growing of the crop and so was induced to collect together all the information then available. Embodying this with the results of his own researches, he produced in 1937 the first edition of a bulletin on Mushroom Growing for the Ministry of Agriculture ; this bulletin became their best seller.

Following his first degree of B.Sc.(Agric.), he was awarded the M.Sc. in 1923, and the D.Sc. in 1935.

Ware followed Professor Salmon as Head of the Mycology Department at Wye College in 1937, and directed research and advisory work through the difficult war

years. The decision to absorb the Department into the Civil Service prompted his retirement in 1946.

Although a capable speaker, as demonstrated by the occasional papers which he read, and by his frequent lectures to students and to farmers, he had an aversion to committee work and did not seek office in the learned societies. Nor was he ambitious for the high academic rewards which could undoubtedly have been his.

In connexion with his work, Ware had visited many European countries as well as the United States ; in retirement he was able to satisfy a taste for travel by occasional journeys abroad. At all times gardens were among his chief pleasures and in retirement gardening was perhaps his main interest.

An unusual degree of modesty and reserve did not prevent the emergence of an engaging and pleasant personality which attracted a wide circle of friends. Except for the years of the first world war, Ware lived in Wye from his student days onwards. His passing will be regretted by villagers as well as by his colleagues.

In 1937 he married Leonora Boulding who survives him.

H.H.G.

MOLYBDENUM AS A PLANT NUTRIENT

V. THE INTERRELATIONSHIPS OF MOLYBDENUM AND NITRATE SUPPLY IN THE CONCENTRATION OF SUGARS, NITRATE AND ORGANIC NITROGEN IN CAULIFLOWER PLANTS GROWN IN SAND CULTURE

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PREVIOUS papers in this series dealt with effects of varied molybdenum and nitrate supplies on growth and on molybdenum content (1) and on chlorophyll and ascorbic acid and related fractions (2) in cauliflower. The parallel changes in sugars and in nitrate and organic nitrogen content are described here.

EXPERIMENTAL

METHODS.

Culture methods and treatments.

Cauliflower plants, variety Majestic, were grown in purified sand using methods previously described (1) to eliminate molybdenum from the cultures.

The plants were given nitrogen as nitrate at two levels : N_1, 6 mg. eq./L ; N_2, 24 mg. eq./L, and molybdenum was given at eight concentrations ranging by approximately tenfold increments from an estimated minimum value of 0·000005 p.p.m. to 4·8 p.p.m. and a maximum concentration of 19·6 p.p.m. Mo. These treatments were commenced at the time of sowing and were continued throughout the experiment.

Sugars.

Total reducing sugars were estimated in macerated alcoholic extracts of tissues by the micro Somogyi method (10, 11), after evaporation of alcohol *in vacuo*, treatment with lead acetate and removal of lead by oxalate. Sucrose was estimated by difference in the total reducing sugar values before and after hydrolysis by invertase or by treatment with NH_2SO_4 for 30 minutes at 65° C. The two methods yielded similar results. The total reducing sugar values found after the hydrolysis treatment were recorded as " total " sugars.

A more detailed examination of sugars and oligo-saccharides separated by chromatographic methods was also made (by S.C.A.) and will be reported separately.

Starch.

Tests at frequent intervals with iodine showed that starch was apparently absent, as it was not detected at any stage during growth.

Nitrogen fractions.

Total nitrogen was determined on dried ground material by the semi-micro Kjeldahl method of Chibnall *et al.* (4). The ammonia produced was estimated after

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distillation from a Markham apparatus into boric acid buffer in an ammonia-free atmosphere, as described by Conway and O'Malley (5). Standards were included in each set of estimations. When nitrate was present, it was included in the estimation of total nitrogen by use of the salicylic acid method of Street *et al.* (12). It was found that at least two hours' treatment at the salicylic and sulphuric acid stage in this method was required. The iron reduction method of Pucher *et al.* (7) gave unsatisfactorily low values.

Nitrate nitrogen was estimated in dry material by phenoldisulphonic acid according to the procedure of Richards and Templeman (9).

Nitrite was determined by the Greiss-Ilosvay procedure (13). Hydroxylamine was estimated as nitrite by difference before and after oxidation with iodine in glacial acetic acid. Ammonia was estimated in alcoholic extracts by the micro-diffusion method of Conway and O'Malley (5), which was found to be more reliable than others tested.

RESULTS.

Sugars.

Leaves were sampled at 60, 88 and 180 days' growth. At the last date roots, stems and curds were also sampled. Results are given in terms of fresh weight.

The changes noted in the various sugar fractions with respect to time, plant part, nitrate level and molybdenum supply appeared to be complex. There were sometimes unexpectedly large differences between concentrations at adjacent molybdenum levels.

Results for sucrose were subject to the sum of errors for total and reducing sugars and were, therefore, the least consistent. As sucrose was also the smallest fraction the variations were relatively greater For these reasons conclusions relating to sucrose are always more tentative than those relating to the other fractions.

Distribution of sugars.

Total and reducing sugars and sucrose showed increasing values in the following order : roots, leaves, stems, curds, at both nitrate levels, except for a low value for sucrose in curds of plants grown with high nitrate. Means of values for all molybdenum levels are given in Table I. All differences for total and reducing sugars were significant ($P < 0{\cdot}01 > 0{\cdot}001$), except that between stems and curds with high nitrate. Differences in concentrations between different organs at the same individual molybdenum level were also significant ($P < 0{\cdot}05 > 0{\cdot}01$). Reducing sugars usually comprised the greater part of the total except in roots.

TABLE I.

Mean values for total and reducing sugars and sucrose as per cent. of fresh weight in 180-day-old plants.

	Roots		Leaves		Stems		Curds	
	Low NO_3	High NO_3	Low NO_3	High NO_3	Low NO_3	High NO_3	Low NO_3	High NO_3
Total	0·10	0·08	0·90	0·77	1·77	1·38	2·65	1·67
Reducing	0·04	0·025	0·75	0·64	1·30	1·20	1·82	1·56
Sucrose	0·06	0·06	0·15	0·15	0·69	0·31	0·83	0·21

Effect of age.

Leaves contained higher concentrations of all sugars at 60 days than at 88 or 180 days in the low nitrate series. There were no appreciable changes with age in the high nitrate series. Means of sugar concentrations for all molybdenum levels at the three ages are given in Table II.

TABLE II.

Mean sugar concentrations in leaves for all molybdenum levels at three ages.

Nitrogen level	60 days		88 days		180 days	
	Total	Reducing	Total	Reducing	Total	Reducing
Low nitrate ..	2·13	1·21	0·74	0·42	0·90	0·75
High nitrate ..	0·74	0·58	0·74	0·46	0·78	0·63

Effect of nitrate supply.

The low nitrate supply resulted in higher concentrations than those produced with high nitrate for total and reducing sugars at all molybdenum levels at 60 days. This effect of nitrate supply was highly significant, $P<0·001$ for reducing sugars, $P<0·01$ for total sugars. The results are shown in Table III.

TABLE III.

Effects of nitrate supply on sugar concentrations in cauliflower leaves at 60 days.

Mo level	0·000005	0·00005	0·0005	0·005	0·05	0·48	4·8	19·2
Total sugars								
N_1	2·36	1·20	0·88	3·37	2·25	3·36	1·95	1·69
N_2	0·39	0·35	0·61	0·70	1·03	1·26	0·83	0·73
Reducing sugars								
N_1	1·49	0·94	0·75	0·94	1·30	1·80	1·48	0·99
N_2	0·39	0·28	0·43	0·67	0·81	0·80	0·59	0·67

Nitrate supply had no significant or consistent effect on sugar concentrations in leaves at 88 days and no consistent effect at 180 days (Fig. 1), although the mean values for all molybdenum levels were significantly greater with low than with high nitrate supply ($P<0·05$). In stems at 180 days the concentrations of total sugars were greater with the low than with the high nitrate supply ($P<0·05$), but reducing sugars were not affected. As there was no molybdenum-nitrogen interaction at this stage the results are shown here as means for both nitrogen series (Fig. 2).

Effect of molybdenum supply.

The concentrations of total and reducing sugars varied in a complex manner with changes in molybdenum supply. The data for 60 days, presented in Table III, and those obtained at 88 days, but not included here, did not show any significant effects for molybdenum. There were, however, possibly maxima in the concentrations of total and reducing sugars at both these times with molybdenum supplies between 0·005

and 0·5 p.p.m. and the apparent decreases in sugar concentrations observed between 0·000005 and 0·0005 p.p.m. Mo at 60 days were consistent in both replicates. At 180 days, the effects of molybdenum supply on total and reducing sugar concentrations in leaves shown in Fig. 1 were highly significant ($P<0·001$). Total and reducing sugar concentrations showed marked maxima at 0·05 p.p.m. Mo ($P<0·01>0·001$). Following the decline from the peak values at 0·05 p.p.m. Mo, there appeared to be,

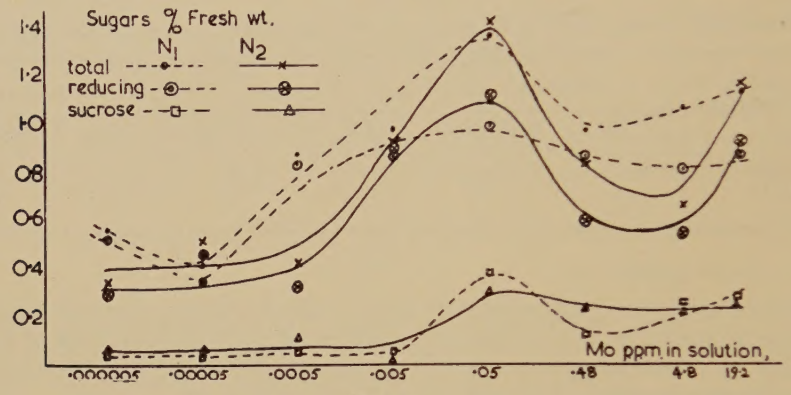

FIG. 1.

Effects of molybdenum and nitrate supply on sugars in cauliflower leaves at 180 days' growth. N_1-low nitrate, N_2-high nitrate. L.S.D. 5 per cent. for Total Sugars 0·28, Reducing Sugars 0·27.

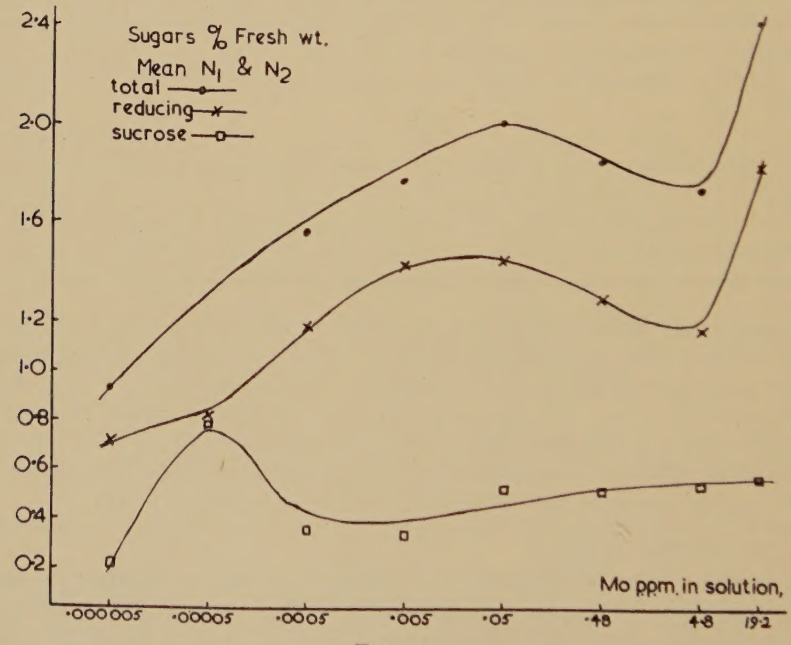

FIG. 2.

Effects of molybdenum supply on sugars in cauliflower stems at 180 days' growth. (Means for both N series). L.S.D. 5 per cent. for Total Sugars 0·68, Reducing Sugars 0·51.

beyond 4·8 p.p.m., a secondary increase in sugar concentrations; $P<0·05$ for total sugars, and $P<0·01$ for reducing sugars in the high nitrate series. There was a significant nitrogen/molybdenum interaction ($P<0·05$) for effects on reducing sugars shown in Fig. 1. A similar interaction possibly occurred for effects on total sugars, but it did not attain significance. Changes in sucrose concentrations were mainly similar to those for total and reducing sugars, but were not significant.

Effects of changes in molybdenum supplies on sugar concentrations in stems resembled somewhat those observed in leaves at 180 days (Fig. 2). The overall effects of molybdenum were significant for total sugars ($P<0·05$) and for reducing sugars ($P<0·01$). The increases in concentrations of reducing sugars between 0·000005 and 0·05 p.p.m. Mo in the low nitrate series and between 0·000005 and 0·005 p.p.m. Mo in the high nitrate series were highly significant ($P<0·01$). Similar changes occurred in total sugars over corresponding molybdenum ranges for the respective nitrate series ($P<0·05>0·01$). As in leaves at 180 days, there appeared to be secondary increases in total and reducing sugar concentrations beyond 4·8 p.p.m. Mo. These were significant; $P<0·001$ for means of both nitrogen levels in the absence of any nitrogen/molybdenum interaction, and $P<0·05$ for individual nitrate series.

Data for sugar concentrations in roots are not presented as they were low and showed no consistent changes with treatments. The data for sugar concentrations in curds at molybdenum levels over the range in which curds were formed are shown in Table IV. There appeared to be progressive increases in concentrations of total sugars and, possibly, reducing sugars with increases in molybdenum supply up to 19·2 p.p.m. in the low nitrate series, but no marked trends occurred in the high nitrate series.

TABLE IV.

Effects of molybdenum and nitrate levels on sugar content (per cent. fresh weight) of curds. 180 days' growth.

NO_3 level	Molybdenum level						
	0·00005	0·0005	0·005	0·05	0·48	4·8	19·2
Total sugars							
N_1	No curd	1·75	1·85	1·90	2·66	2·73	5·01
N_2	1·42	1·61	2·06	1·55	1·75	1·92	2·04
Reducing sugars							
N_1	No curd	0·94	1·64	0·77	2·10	1·98	3·49
N_2	1·25	1·42	1·85	1·35	1·64	1·56	1·83

NITROGEN FRACTIONS.

Leaves were sampled for nitrogen estimations at 34, 65, 90 and 182 days' growth. At the last stage roots, stems and curds were also sampled. The dates were slightly different from those at which material was sampled for sugars as simultaneous sampling was impracticable. The results are expressed on a dry-weight basis, since the determinations of nitrate and total nitrogen were made on dried material.

The concentrations found for nitrite, hydroxylamine and ammonia in fresh material were low in all samples and showed no consistent effects of any treatments. These fractions are, therefore, not considered further.

Nitrate.

Nitrate was almost always present in leaves in appreciable quantities even at normal or high molybdenum levels. Stems contained less nitrate than leaves ; roots contained very little and curds had negligible concentrations, usually below 0·01 per cent. of dry weight.

At deficiency levels of molybdenum, nitrate concentrations in leaves were abnormally high (Figs. 3-6) and at 65, 90 and 182 days' growth they decreased rapidly with molybdenum increase up to 0·005 p.p.m. Beyond this molybdenum level changes were small. Except for one value, for 0·00005 p.p.m. Mo at 90 days, the concentration of nitrate in the leaf was higher with the high nitrate supply at all molybdenum levels, including those at which there was no further response to molybdenum ($P<0·01>0·001$). At the first and last sampling dates (at 34 days, Fig. 3, and at 182 days, Fig. 6) the fall in the nitrate concentration as molybdenum increased was more gradual in the high than in the low nitrate treatments, and continued up to 0·005 p.p.m. Mo. This difference in drift between the high and low nitrate series was not obvious at the other two sampling dates.

There were only small changes in the nitrate content of stems of plants due to changes in molybdenum level in the high nitrate treatments ; but a sharp decrease occurred between 0·000005 and 0·00005 p.p.m. Mo in the low nitrate series. The data are not presented here.

Organic Nitrogen.

Since inorganic fractions other than nitrate were found to be negligible in amount, organic nitrogen was estimated as the difference between total nitrogen and nitrate nitrogen. There were increases in organic nitrogen content of leaves with increasing molybdenum supply, up to approximately 0·05 p.p.m. Mo at 34, 65 and 182 days' growth (Figs. 3, 4, 6) with both high and low nitrate supplies. Beyond this molybdenum level there were usually decreases. The changes were more regular in plants given the high nitrate supply.

The overall effects of molybdenum on organic nitrogen content at these times were highly significant ($P<0·001$). There was no significant effect of nitrate level ; but the nitrogen/molybdenum interaction was significant at 65 days ($P<0·05$) and at 182 days ($P<0·01$), suggesting that the curves prepared from data for the low and high nitrate series at these times do not show a similar trend at all molybdenum levels. Differences between maxima and the lower concentrations at the highest molybdenum levels were significant in the low nitrate series ($P<0·05$), but were not usually so in the high nitrate series.

At 90 days' growth and at both nitrate levels (Fig. 5) the peak content was reached at a much lower molybdenum level and the maximum appeared to be more sharply defined. At this date also, leaves of plants having the high nitrate supply had greater organic nitrogen concentrations at all molybdenum levels. The effects of molybdenum and of nitrate supply were both highly significant ($P<0·001$) at this time, and there was no nitrogen/molybdenum interaction.

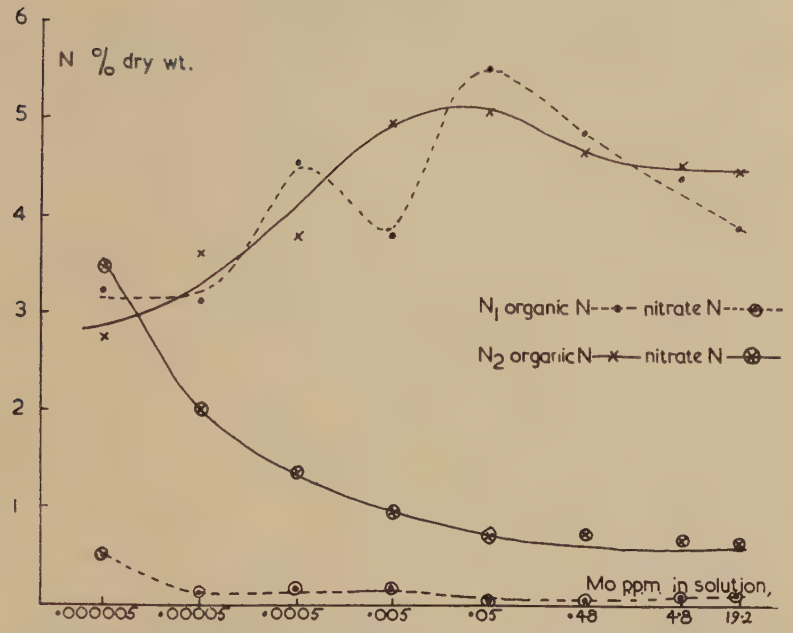

Fig. 3.

Effects of molybdenum and nitrate supply on nitrate and organic nitrogen content in cauliflower leaves at 34 days' growth. N_1-low nitrate, N_2-high nitrate. L.S.D. 5 per cent. for Organic N 0·83.

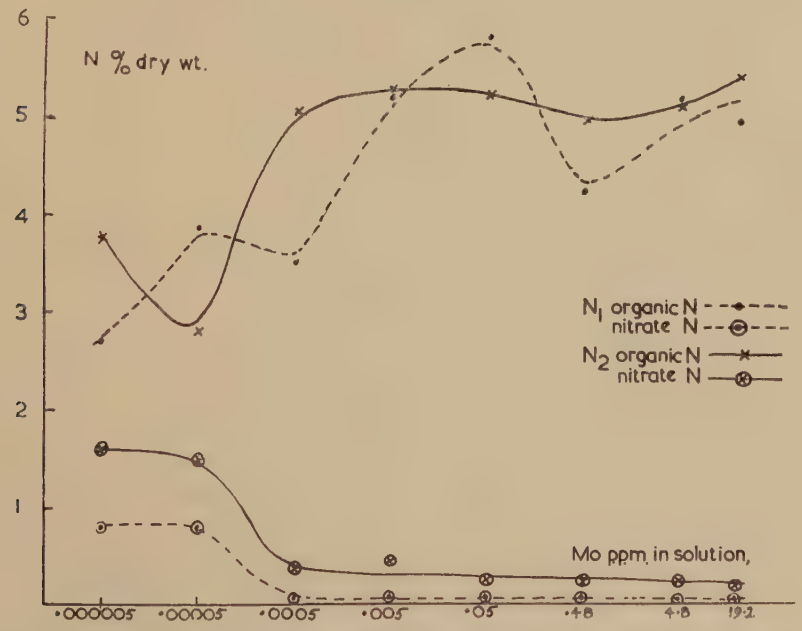

Fig. 4.

Effects of molybdenum and nitrate supply on nitrate and organic nitrogen content in cauliflower leaves at 65 days' growth. N_1-low nitrate, N_2-high nitrate. L.S.D. 5 per cent. for Organic N 1·01.

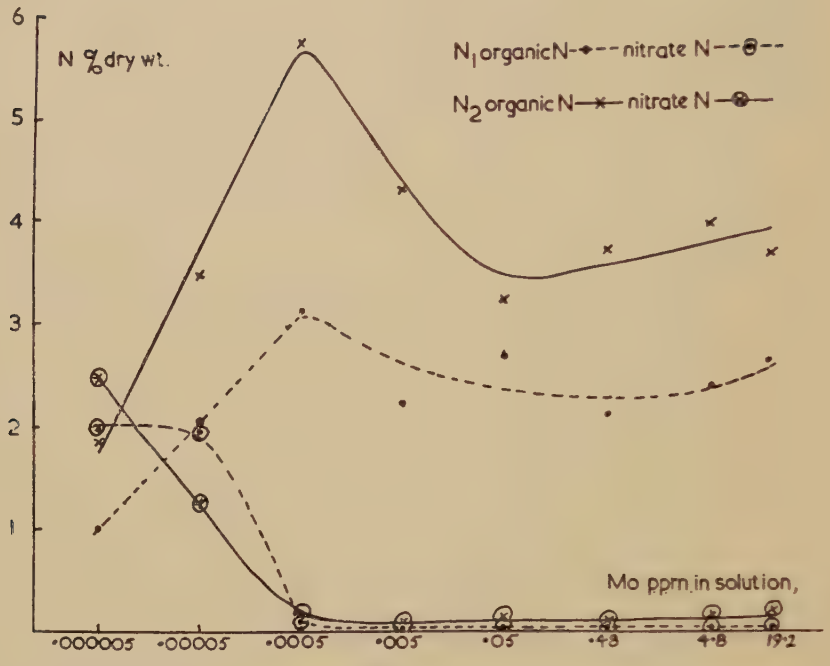

FIG. 5.

Effects of molybdenum and nitrate supply on nitrate and organic nitrogen content in cauliflower leaves at 90 days' growth. N_1-low nitrate, N_2-high nitrate. L.S.D. 5 per cent. for Organic N 1·30.

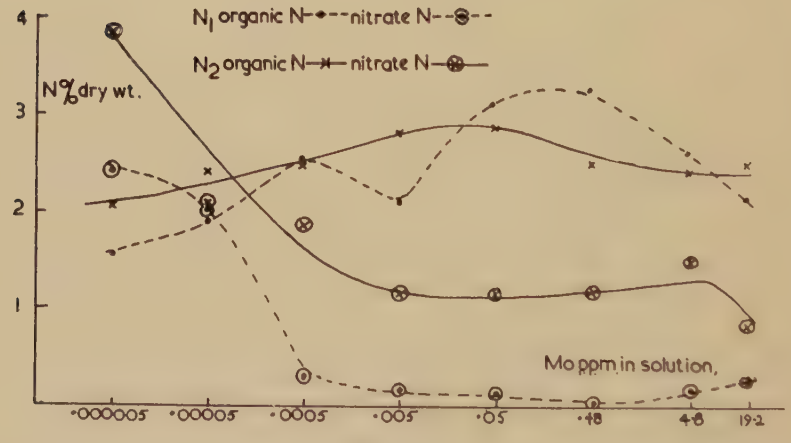

FIG. 6.

Effects of molybdenum and nitrate supply on nitrate and organic nitrogen content in cauliflower leaves at 182 days' growth. N_1-low nitrate, N_2-high nitrate. L.S.D. 5 per cent. for Organic N 0·45.

Data for the organic nitrogen content of stems are not shown here. There appeared to be slight increases with increase in molybdenum level, but the response was not statistically significant.

Total Nitrogen Content per Plant.

Fig. 7 shows organic N per plant together with the proportion of the total represented by nitrate. With low nitrate supply the amount of organic nitrogen produced per plant did not increase beyond 0·0005 p.p.m. Mo and the ratio of nitrate to total nitrogen fell to a constant value. With the high nitrate supply, the total organic nitrogen produced increased steadily up to 0·005 p.p.m. Mo and showed some increase, though at a reduced rate, beyond this level. The ratio of nitrate to total

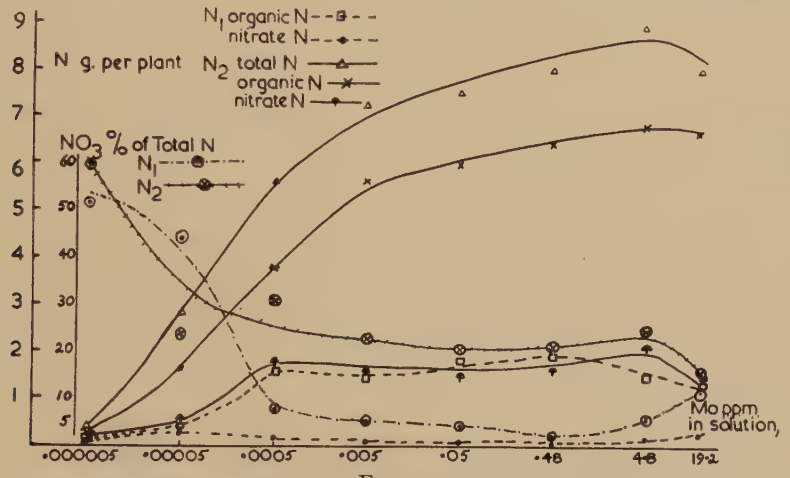

Fig. 7.

Effect of molybdenum and nitrate supply on the total nitrogen content per plant at 182 days' growth and on the proportion of nitrate in the total. N_1-low nitrate, N_2-high nitrate. L.S.D. 5 per cent. for Organic N 1·72.

nitrogen per plant decreased to 0·005 p.p.m. and then remained fairly steady. From 0·0005 p.p.m. Mo upwards, the proportion of nitrate N remained substantially greater in the high than in the low nitrate series. The overall effects of molybdenum and of nitrate level were highly significant ($P<0·001$). The molybdenum/nitrogen interaction was also highly significant ($P<0·01$). Inspection of the data illustrated in Fig. 7 suggests that this interaction arises from the differences between the molybdenum levels beyond which further rapid increase in total organic nitrogen did not occur. The total organic nitrogen appeared to increase with increased molybdenum supply beyond 0·005 p.p.m. Mo in the high nitrate series, but the difference between that at 0·005 p.p.m. and the maximum was not significant.

The nitrate content per plant increased for plants in the high nitrate series up to 0·005 p.p.m. Mo, beyond which there was no appreciable change. In the low nitrate series there was a possible slight increase up to 0·00005 p.p.m. Mo followed by a decrease.

Data for total nitrogen per plant are also shown for the high nitrate series, but are omitted for the low nitrate series to avoid confusion.

DISCUSSION.

The role of molybdenum in nitrate reduction is generally accepted and its relation to the transformation of nitrate to organic nitrogen appears clearly in the present results. The quantitative molybdenum requirement for this role is apparently nearly satisfied at 0·0005 p.p.m. Mo in the nutrient with low nitrate supply (Figs. 4, 5, 6) and usually, at this concentration (Figs. 4, 5) or at 0·005 p.p.m. Mo, with the high nitrate supply (Figs. 3, 6). This is because organic nitrogen per plant (Fig. 7) increases not at all, or only slightly, and the rapid decrease in accumulated nitrate is mainly completed at such levels of molybdenum ; these also correspond to the levels beyond which there were no significant increases in growth (1).

It was shown in a previous paper (2) that molybdenum supply affected the contents of ascorbic acid and chlorophyll in cauliflower plants grown in the same experiments as are described here. It was concluded from these results (2) that the increased contents of ascorbic acid resulting from increased molybdenum supplies up to 0·005 p.p.m. were not directly caused by increased chlorophyll contents which were also produced. The possibility that these changes in ascorbic acid may be due to changes in sugar concentrations observed here must be considered, since other investigations (3, 6, 8) have shown that sugar concentration may be a determining factor in ascorbic acid production. Comparisons of the curves relating ascorbic acid content and molybdenum supply (2) with those obtained here for sugars show, however, no evidence of a causal relationship. Thus whereas, at all sampling dates, ascorbic acid content reached a maximum at about 0·0005 p.p.m. Mo for both nitrate series and then remained mainly steady up to 19·2 p.p.m. Mo, there were either large fluctuations in sugar concentrations over the same molybdenum range, or the molybdenum levels producing maximum sugar concentrations were normally much higher than those producing the maximum ascorbic acid. In addition there was no correspondence between the high concentrations of sugars in curds and the low concentrations of ascorbic acid in the same regions (2). The effects of molybdenum supply on sugar concentrations do not, therefore, afford an obvious explanation of the effects found with ascorbic acid.

A relationship between molybdenum supply and sugar content was disclosed ; but the effects of changes in molybdenum supply on sugar concentrations at different ages show that this relationship may be complex and possibly indirect in nature. Thus, the range of increase in molybdenum supply over which increased sugar concentrations occurred was generally much greater than the range over which major changes in growth (1), ascorbic acid concentrations (2) or nitrate reduction were observed, especially at the lower nitrate level. There was often no correspondence between changes in sugar concentrations noted here and those in chlorophyll content recorded earlier (2) over the range of molybdenum levels tested. In instances where there was some apparent similarity in these changes, the molybdenum levels producing maximum sugar concentrations were often higher than those producing maximum chlorophyll content, indicating that molybdenum supply still affected sugar content at levels that no longer affected chlorophyll content as a limiting factor.

It was reported (1) that the onset of whiptail symptoms in young leaves was associated with an apparently abnormal condition of the middle lamella of groups of cells. This early whiptail condition may have resulted from impaired pectin metabolism and it is possible that pectic compounds may be affected by molybdenum supply in a similar manner to that found for sugars. The low concentrations of

sugars found over the range 0·000005-0·0005 p.p.m. molybdenum, especially in the high nitrate series which was associated with the earliest and most severe occurrence of whiptail (1) at about 60 days, are, therefore, particularly noteworthy. On the other hand the lower nitrate supply limited growth even at the lowest molybdenum levels (1) and the plants also contained lower concentrations of accumulated nitrate. Under these conditions the demands on available carbohydrate supplies may have been decreased to an extent producing the apparent, though not significant, increase in sugar concentration at the lowest molybdenum levels at 60 days (Table III) and correspondingly decreasing the occurrence of whiptail (1) observed in these treatments.

Further evidence regarding points discussed here and observations on the effects of molybdenum supply on growth and composition of the cauliflower, given different sources of nitrogen, will be presented in subsequent papers.

SUMMARY.

1. Cauliflower plants were grown with two levels of nitrate (6 and 24 mg. eq./L) and with eight levels of molybdenum, ranging from 0·000005 to 19·2 p.p.m. Leaves, stems and other parts were analysed at intervals for sugars and for nitrogen fractions.

2. Stems and curds were rich in sugars and appeared to contain more than half the total sugar content of mature plants. Starch was not detected.

3. Concentrations of total and reducing sugars in leaves and stems of mature plants increased significantly with increased molybdenum supply up to about 0·05 p.p.m. Mo and then usually decreased. There were further increases at the highest molybdenum levels. Sucrose showed smaller but mainly similar changes.

4. High nitrate supply resulted in decreased total and reducing sugar concentrations in leaves at 60 days and in decreased total sugars in stems of mature plants.

5. Nitrate nitrogen concentrations decreased sharply with small increases in molybdenum level up to 0·0005 p.p.m. Mo and showed little change at higher levels.

6. There were no notable changes in small concentrations of nitrite, hydroxylamine or ammonia.

7. Organic nitrogen concentration increased with molybdenum supply. The maxima usually occurred at molybdenum levels similar to, or slightly higher than, those beyond which there were only slight or negligible decreases in nitrate concentration. These levels were usually lower than those associated with maximum sugar contents.

8. Comparisons of the present results with those presented previously suggest that changes in sugar concentrations found here do not directly account for the effects of molybdenum on ascorbic acid content already noted.

9. It is tentatively suggested that the low sugar concentrations found at 0·000005-0·0005 p.p.m. Mo, especially in the high nitrate series, may be related to or parallel to changes in constituents of the middle lamella, previously noted to appear abnormal in localized areas of leaves affected by whiptail.

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MOLYBDENUM AS A PLANT NUTRIENT

VI. EFFECTS OF MOLYBDENUM SUPPLY ON THE GROWTH AND COMPOSITION OF CAULIFLOWER PLANTS GIVEN DIFFERENT SOURCES OF NITROGEN SUPPLY IN SAND CULTURE

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PREVIOUS papers have described the effects of molybdenum on the growth (3) and composition (4, 5) of cauliflower plants grown with two levels of nitrate nitrogen. It was found that molybdenum deficiency resulted in decreased contents of chlorophyll, ascorbic acid, sugars and organic nitrogen. This paper describes results obtained with cauliflower plants grown with nitrogen as nitrate, nitrite, ammonium compounds, urea or glutamic acid. The intention was to compare the effects of molybdenum on growth and composition under different conditions of nitrogen nutrition. The results were obtained in two main experiments carried out in 1951 and 1952, of which the first was briefly noted (1), and in other experiments in 1953-54 in which additional confirmation of certain points was obtained.

EXPERIMENTAL.

METHODS.

Culture methods.

Cauliflower plants, variety Majestic, were grown in purified sand (8), contained in 2-gallon pyrex glass pots for molybdenum deficiency levels and in 10-inch or 12-inch bitumen-painted clay pots for normal and excess molybdenum levels.

In order to avoid effects of injury from ammonium sulphate, to which cauliflower grown in sand culture is susceptible, in this treatment calcium carbonate was mixed with the sand in 1951 and 1952. An additional nitrate treatment, with calcium carbonate added, was included to determine the possible effects of calcium carbonate. In 1953 and 1954, calcium carbonate was included in all treatments. The calcium carbonate was prepared by precipitation on mixing calcium chloride and sodium carbonate solutions, previously purified by the copper sulphide method (8, 12), and was thoroughly washed with water. 70 g. calcium carbonate were added to each 2-gallon or 12-inch pot and 50 g. to a 10-inch pot. Ammonium citrate or malate was also given, but less successfully, to provide a non-toxic ammonium nitrogen supply. Nitrate plus citrate or malate treatments were also included as checks on the effects of the organic acid supplements.

The nutrient solutions were prepared from stock solutions which were purified by twofold copper sulphide precipitation (12) under the conditions standardized for use at Long Ashton (8). It was found that phosphates, sodium carbonate and organic

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compounds, such as glucose, amino acids, organic acid salts and urea could all be treated successfully in this manner. In some instances the copper sulphide remained in a colloidal form. This was precipitated by various methods including more prolonged heating, addition of a few drops of hydrochloric acid, repeated filtration or further copper sulphide precipitation. Alkaline phosphate and carbonate solutions retained some polysulphide after treatment. This was removed by adding copper sulphate, a few drops at a time with stirring, to the hot solution until the yellow colour disappeared. The sulphide precipitate and copper phosphate or carbonate were removed by filtration on sintered pyrex glass filters. The sodium nitrite solution was adjusted to pH 7·5-8·0 before treatment with copper sulphate and hydrogen sulphide to avoid decomposition of nitrite by acidification during treatment. With care no interference from polysulphide was encountered. Glutamic acid was dissolved in sodium hydroxide solution containing about one-half the respective equivalent of sodium hydroxide and was stored in the refrigerator after purification.

Purified ferric citrate was prepared as described (8) from citric acid, treated by the copper sulphide method, and ferric chloride. The latter was either treated exhaustively with hydrogen sulphide, after addition of copper sulphate and dilute hydrochloric acid, or was prepared by first extracting ferrous sulphate in 6N hydrochloric acid with ether, oxidizing the iron to the ferric state and extracting ferric chloride with ether from 6N hydrochloric acid (8).

Nitrogen was given at 10 mM/l in 1951 and at 12 mM/l in 1952-54. The sources of nitrogen used were as follows:

1951 : nitrate, nitrate plus citrate, nitrite, ammonium sulphate, ammonium nitrate, ammonium citrate, urea.

1952 : nitrate, nitrate plus citrate, malate or glucose ; nitrite, nitrite plus citrate or malate ; ammonium sulphate or citrate or malate, ammonium sulphate plus glucose ; urea, urea plus citrate or malate ; ammonium nitrate, ammonium nitrite (each 6 mM/l) ; nitrate plus arginine (1 mM/l) ; glutamic acid.

1953-54 : nitrate, nitrite, ammonium sulphate, ammonium nitrate, ammonium nitrite, urea (except in 1954).

The concentrations of the other macro- and micronutrient elements were those given in the usual Long Ashton nutrient solution (8). It was necessary to give calcium as chloride and potassium as sulphate throughout to provide uniform conditions.

The following molybdenum levels were given :

" Nil " estimated to provide 0·000005-0·00001 p.p.m. Mo from the glass distilled water or resin purified rainwater (8) ; a threshold deficiency level of 0·00005 p.p.m. ; normal levels of 0·05 or 0·5 p.p.m. ; and a toxic level of 50 p.p.m. given in 1951.

In 1951 NaH_2PO_4 was used to provide phosphorus in the ammonium nitrate and ammonium sulphate treatments, but this resulted in the production of low pH values (about 3·5) during growth. In 1952 Na_2HPO_4 was used in all the ammonium treatments, including ammonium nitrite, and a mixture of the two phosphates in equal proportions was used for the nitrite treatments. The finely divided precipitate of calcium phosphate produced was readily available to the plants under sand culture conditions and also avoided the production of crystalline calcium sulphate in the ammonium sulphate medium.

There was evidence of some microbiological activity in 1952 in nutrient solutions containing ammonium citrate or malate or urea plus citrate or malate.

Plants were sprayed regularly with nicotine solution, D.D.T. suspension or derris dust and inspected daily for caterpillar attack. Pest control methods were generally efficient and did not interfere with the treatments.

There were four pots in each treatment in 1951 and 1952, divided between two randomized blocks, eight pots per treatment in 1953 and five pots in 1954. Several seeds were sown and seedlings were thinned out at frequent intervals until two plants were left in each pot, except in some of the more severely affected treatments where four or five plants were left. In 1953 and 1954 seeds were sown in a nitrate medium and transplanted 10 days later into the respective treatments.

RESULTS.

Visual symptoms.

As visual symptoms are important criteria of molybdenum deficiency they are dealt with in some detail.

(1) *Normal molybdenum supply (0·05-0·5 p.p.m.).*

Plants grown at these levels were mainly normal in appearance except for some effects of ammonia injury in certain treatments referred to below. In the absence of calcium carbonate the ammonium sulphate treatment was lethal and ammonium nitrite was unsatisfactory. When calcium carbonate was given these treatments permitted normal growth. In 1951 the nitrite treatment resulted in marked anthocyanin pigmentation in the central areas of leaves of some plants.

(2) *Molybdenum deficiency (0·000005-0·00005 p.p.m.).*

The effects referred to as primary symptoms of molybdenum deficiency in cauliflower, namely bright mottling, marginal cupping and wilting, scorching and withering and the sequence of secondary symptoms associated with whiptail have been described (3, 11). The secondary symptoms referred to here are grouped as follows : *Slight :* localized interveinal chlorosis, necrosis, perforation and enlarged irregular holes produced during expansion of young leaves. *Moderate :* marginal necrosis of very young leaves leading to defective and distorted laminae. *Intense :* total loss of lamina, death of rudimentary leaves and death of the growing point.

The symptoms produced by molybdenum deficiency are shown in Table I in relation to the general colour, incidence of primary symptoms and intensity of the secondary symptoms comprising the whiptail syndrome. Differences observed in different years are noted where necessary. It is apparent from Table I that primary symptoms were confined to treatments containing nitrate. Whiptail symptoms occurred in *all* treatments, but with different intensity. They were usually most intense in nitrate treatments, either after partial recovery from the primary symptoms or at the 0·00005 p.p.m. Mo level, as recorded previously (3, 11), and least intense with ammonium nitrite (except in 1954). Plants given nitrate alone remained at the primary symptom stage when the molybdenum supply was successfully maintained close to the estimated minimum concentration of 0·000005 p.p.m. Mo. Examples of whiptail effects are shown for different nitrogen treatments in Plate I.

(3) *Molybdenum excess (50 p.p.m.).*

The main symptoms were production of blue or golden-brown pigments (3, 14), and chlorosis. Blue pigment production increased in intensity and extent in the order : nitrate and ammonium sulphate with calcium carbonate < nitrate < urea. In the nitrite treatment the distribution was similar to that of the purple anthocyanin pigment seen at the normal molybdenum level. Blue pigment also appeared in the sand in the urea treatment. Plants given ammonium nitrate produced golden-brown pigments. Those given ammonium sulphate without calcium carbonate were acutely chlorotic. The injury from excess molybdenum increased in the order : nitrate < ammonium sulphate with calcium carbonate < ammonium nitrate and nitrite < urea and ammonium sulphate without calcium carbonate.

(4) *Ammonia injury.*

Symptoms associated with ammonia injury, namely blue-grey and sometimes yellow colouration in irregular areas within green margins of older leaves, followed by grey necrotic patches and marginal scorching, occurred in 1951 and 1952 in some ammonium and urea treatments. They appeared to bear some relationship to molybdenum supply in 1951 as shown below :

Relation of "ammonia injury" to molybdenum supply.

	$(NH_4)_2SO_4$ (with $CaCO_3$)	NH_4 Citrate	NH_4 Malate	NH_4NO_3	NH_4NO_2	Urea	Urea + Citrate	Urea + Malate
+Mo	nil	nil	trace	trace	nil	nil	nil	nil
−Mo	nil	moderate to intense	moderate	trace	trace	slight	trace	nil

YIELDS.

1951 Experiment.

Plants were removed after 42, 80 and 148 days' growth and dried. Yields are shown in the histograms in Fig. 1 with the differences for significance at the 5 per cent. level.

Yields were decreased by molybdenum deficiency at all three stages of growth. There was, however, some evidence to suggest that the normal level of 0·5 p.p.m. Mo was above the optimum at 42 days for some nitrogen treatments, e.g. ammonium sulphate or urea. Differences between yields at 0·000005-0·00001 p.p.m. or 0·00005 p.p.m. and those at 0·5 p.p.m. Mo were significant ($P<0·05$) for all individual nitrogen treatments at 148 days' growth. Differences between yields at 0·00001 and 0·00005 p.p.m. Mo were also significant for nitrate, nitrate plus calcium carbonate, nitrate plus citrate and ammonium sulphate plus calcium carbonate at 148 days' growth. Acute injury was caused by excess molybdenum at 50 p.p.m. in the presence of urea or ammonium citrate. The somewhat low yields at 0·5 p.p.m. Mo in these treatments may also have been due to molybdenum excess effects, as growth in 1952-54 with 0·05 p.p.m. molybdenum was outstandingly better in the presence of urea and ammonium compounds.

TABLE I.

Effects of molybdenum deficiency on visual symptoms in cauliflower plants grown with different sources of nitrogen.

Nitrogen treatments and supplements	0·000005-0·00001 p.p.m. 1951, 1952, 1954			0·00005 p.p.m. 1951 and 1953		
	Colour, etc.	Primary symptoms	Whiptail syndrome	Colour, etc.	Primary symptoms	Whiptail syndrome
Nitrate 1951-54	Chlorotic	Moderate to very intense, often lethal	Intense when following partial recovery in some plants	Pale green	Slight to moderate	Moderate to intense
Nitrate plus citrate 1951-52	Chlorotic	Intense to very intense, lethal	Intense when following partial recovery in some plants	Pale green	Slight to moderate	Moderate to intense
Nitrate plus malate 1952	Chlorotic	Very intense, lethal	Plants remained at primary stage	—	—	—
Nitrate plus sucrose 1952	Chlorotic	Intense	Intense, following partial recovery in some plants	—	—	—
Nitrate plus arginine 1952	Chlorotic	Moderate	Moderate	—	—	—
Nitrate plus nitrite 1952	Chlorotic	Moderate	Moderate to intense	—	—	—
Nitrite 1951-54	Normal green	Faint grey mottle in seedlings	Nil 1951 Slight to moderate 1952 Moderate 1954	Normal green	Nil	Nil 1951 Moderate 1953
Nitrite plus citrate 1952	Normal green	Faint grey mottle in seedlings	Moderate	—	—	—
Nitrite plus malate 1952	Normal green	Faint grey mottle in seedlings	Slight to moderate	—	—	—
Ammonium sulphate 1951-54	Dark green	Nil	Slight 1951 Moderate to intense 1952-54	Dark green	Nil	Nil 1951 Moderate 1953
Ammonium citrate 1951-52	Dark green	Nil	Moderate	Dark green	Nil	Slight
Ammonium malate 1952	Dark green	Nil	Moderate	—	—	—
Ammonium nitrate 1952-54	Initially mottled, later green 1951 Mottled 1952-54	Moderate to intense wilting, scorching and marginal cupping	Moderate to intense	Mainly dark green, some mottling	Slight	Moderate 1951 Moderate to intense 1953
Ammonium nitrite 1952-54	Dark green	Nil	Slight 1952 Moderate 1954	Dark green	Nil	Slight 1953
Urea 1951-53	Normal green	Occasional faint mottle	Slight to moderate	Dark green	Nil	Slight
Urea plus citrate 1952	Normal green	Nil	Moderate	—	—	—
Urea plus malate 1952	Normal green	Nil	Moderate	—	—	—
Glutamic acid 1952	Dark green	Nil	Moderate to intense	—	—	—

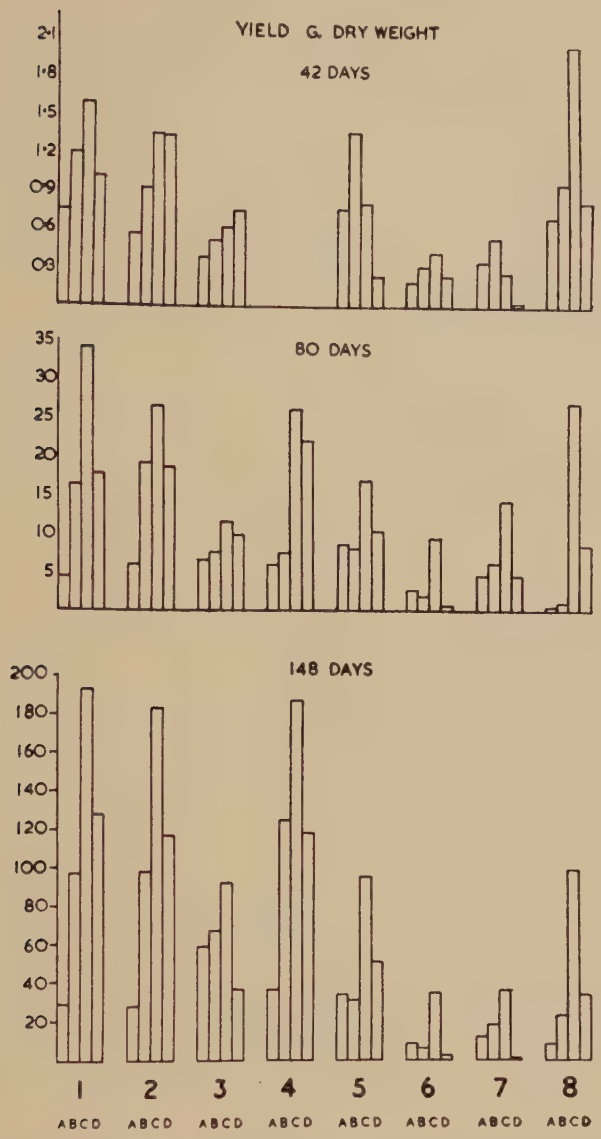

Fig. 1.

Effects of molybdenum level and source of nitrogen on yields (as g. dry wt. per plant) of cauliflower grown in 1951.

Mo level : A, Mo_1 0·000005 p.p.m. ; B, Mo_2 0·00005 p.p.m. ; C, Mo_3 0·5 p.p.m. ; D, Mo_4 50 p.p.m.

N source : 1 Nitrate. 2 Nitrate with added Calcium carbonate.
3 Nitrite. 4 Nitrate with added Citrate.
5 Ammonium sulphate with added Calcium carbonate. 6 Ammonium citrate.
7 Urea. 8 Ammonium nitrate.

Differences for significance at 5 per cent. level :
42 days, 1·02 ; 80 days, 11·72 ; 148 days, 19·00 g.

1952 Experiments.

Yields at 116 days are given in Table II. Molybdenum deficiency resulted in decreased yields for all nitrogen treatments and the general molybdenum effect was significant ($P<0.01$). The relative effects were greatest for the nitrate group, with the exception of nitrate plus arginine and nitrate plus nitrite, but including ammonium nitrate. The nitrite group were next in order of relative growth decrease, with the exception of ammonium nitrite. Decreases with ammonium sulphate or malate were slightly less, followed by the urea group and lastly by glutamic acid and ammonium citrate. Individual molybdenum effects were significant ($P<0.05$) for all nitrate treatments, ammonium nitrate, nitrite, nitrite plus citrate and ammonium sulphate plus calcium carbonate. Differences with urea plus citrate just failed to attain significance. Differences due to molybdenum for the other nitrogen treatments were below significance, probably due partly to there being only a single degree of freedom for Mo and to the somewhat low yields as a whole in some nitrogen treatments.

The molybdenum/nitrogen treatment interaction was highly significant ($P<0.01$) indicating that the molybdenum requirement varied considerably with nitrogen source. Inspection indicated that the interaction was in fact due to the contrast between the nitrate and non-nitrate types of nutrient.

Molybdenum content.

Molybdenum content in leaves was estimated at 80 and 148 days' growth in 1951 and at 116 days in 1952. Results are given in Tables II and III. In 1951, plants grown in the ammonium sulphate, ammonium citrate, ammonium nitrate or urea treatments with 50 p.p.m. Mo contained higher molybdenum concentrations than the others at 80 days' growth. This effect was found only in the urea and possibly in the ammonium nitrate treatments at 148 days. The effect was smaller or absent at the lower molybdenum levels. Plants given nitrate or nitrate plus calcium carbonate usually contained less molybdenum than the others when grown with 0·5 or 50 p.p.m. Mo.

In 1952, there were no consistent or marked differences in molybdenum content of plants given different nitrogen treatments with 0·05 p.p.m. Mo. The addition of citrate had no consistent effect on the molybdenum content in either experiment.

Molybdenum deficient plants contained 0·03 to 0·14 p.p.m. and 0·06 to 0·23 p.p.m. Mo, when given 0·00001 and 0·00005 p.p.m. Mo respectively in 1951, and 0·01 to 0·1 p.p.m. Mo, when given approximately 0·000005-0·00001 p.p.m. Mo in 1952. These values are consistent with previous results (3) for molybdenum deficiency status.

Chlorophyll.

It was evident visually in 1951 that appreciable loss of chlorophyll occurred only in treatments in which nitrate was given. This observation was confirmed in 1952 by chlorophyll estimations, results of which are given in Table II. The molybdenum/nitrogen interaction was highly significant ($P<0.001$). This was due to the great depression of chlorophyll with molybdenum deficiency in the presence of nitrate, as compared with only small decreases or even increases observed with the other nitrogen sources. In order to test the data further the results of treatments containing the non-nitrate sources only were re-examined in groups comprising each main nitrogen

Table II.

Effects of molybdenum supply and nitrogen source on yield (g. dry wt.) ; on chlorophyll content (mg. per 100 g. fresh wt.) ; on nitrate content (per cent. in fresh wt.) and molybdenum content (p.p.m.), in dry foliage of cauliflower plants grown in sand culture in 1952.

Nitrate source and supplements	Yields		Ratio per cent.	Chlorophyll		Ratio per cent.	Nitrate		Molybdenum content	
	0·00001 p.p.m. Mo	0·05 p.p.m. Mo		0·00001 p.p.m. Mo	0·05 p.p.m. Mo		0·00001 p.p.m. Mo	0·05 p.p.m. Mo	0·00001 p.p.m. Mo	0·05 p.p.m. Mo
Nitrate	22·6	218·2	10·4	111·0	208·7	53·1	0·485	0·053	0·064	3·83
Nitrate+$CaCO_3$	39·1	160·9	24·2	124·7	234·8	51·1	0·502	0·058	0·028	8·84
Nitrate+Citrate	4·9	131·7	3·7	49·9	191·2	26·2	1·375	0·045	0·043	1·30
Nitrate+Malate	1·9	83·2	2·3	49·6	194·9	25·5	1·756	0·072	0·065	3·25
Nitrate+Sucrose	7·0	101·9	6·9	66·6	170·5	39·0	0·960	0·036	0·011	3·60
Nitrate+Arginine	116·2	202·3	57·3	116·0	227·0	51·1	0·332	0·086	0·011	4·19
Nitrate+Nitrite	81·3	155·6	52·2	183·7	223·7	82·2	0·290	0·047	0·033	3·61
Nitrite	54·7	145·2	37·6	188·5	215·1	87·5	0·051	0·046	0·014	5·00
Nitrite+Citrate	27·8	112·0	24·8	190·9	230·8	80·8	0·024	0·014	0·095	4·01
Nitrite+Malate	36·3	99·3	36·5	177·5	190·0	93·3	0·033	0·017	0·054	6·47
Ammonium nitrate	19·1	127·0	15·0	129·5	253·5	51·2	0·214	0·029	0·065	2·60
Ammonium nitrite	24·8	56·4	49·2	272·4	215·9	126·2	0·028	0·005	0·044	2·23
Ammonium sulphate+Calcium carbonate	77·2	177·6	43·4	207·1	198·4	104·2	0·027	0·046	0·072	2·60
Ammonium citrate	50·1	70·7	70·8	233·5	159·6	146·0	0·024	0·043	0·062	1·14
Ammonium malate	29·1	95·9	20·4	228·8	194·6	117·7	0·016	0·023	0·053	3·32
Urea	38·5	85·4	45·1	202·3	235·5	85·8	0·008	0·008	0·028	4·56
Urea+Citrate	50·1	129·1	38·8	198·8	190·4	103·5	0·029	0·018	0·070	4·88
Urea+Malate	45·7	87·9	51·8	191·1	219·2	87·1	0·018	0·031	0·024	5·00
Glutamic acid	44·5	68·9	64·6	230·9	190·7	116·8	0·035	0·004	0·100	8·46
Mean	40·6	123·2		166·5	207·6		0·326	0·036	0·049	4·14

L.S.D in Yield (effect of Mo): $P<0·05$, 43·46 ; $P<0·01$, 58·24 ; $P<0·001$, 76·65 g.

TABLE III.

Molybdenum content (p.p.m. in dry matter) of cauliflower plants grown with different nitrogen and molybdenum supplies in sand cultures in 1951.

Nitrogen supply	80 days				148 days			
	0·00001	0·00005	0·5	50 p.p.m. Mo	0·00001	0·00005	0·5	50 p.p.m. Mo
Nitrate	0·11	0·12	9·99	454	0·04	0·11	6·67	834
Nitrate+Calcium carbonate	0·14	0·07	8·65	434	0·04	0·08	5·70	700
Nitrate+Citrate	0·12	0·09	5·93	321	0·10	0·06	27·93	1,023
Nitrite	0·12	0·10	13·71	587	0·06	0·08	11·99	1,082
Ammonium sulphate+Calcium carbonate ..	0·05	0·12	21·21	1,543	0·09	0·15	17·07	994
Ammonium citrate	0·07	0·23	13·4	1,600	0·03	0·15	15·93	1,148
Ammonium nitrate	0·07	0·15	15·10	1,707	0·06	0·07	15·53	1,404
Urea	0·14	0·11	33·93	2,340	0·13	0·10	19·80	2,426

type. There were no significant effects due to molybdenum and no interactions. It is concluded that appreciable reduction of chlorophyll occurred only when nitrate had been given. Inspection of individual results suggested that the ammonium nitrate treatment produced similar results to nitrate.

Ascorbic and dehydroascorbic acids.

Estimations were made at 33, 76 and 101 days' growth in 1951 and results are shown in the histograms in Fig. 2.

The effects of molybdenum supply on changes in ascorbic acid content of plants given the various nitrogen sources are summarized below in terms of levels of significance :

33 days :

Mo_3 (0·5 p.p.m.)>Mo_1 (0·000005 p.p.m.) with all N sources ($P<0·001$), except ammonium sulphate ($P<0·05$).

Mo_2 (0·00005 p.p.m.)>Mo_1 with ammonium nitrate or urea ($P<0·01$), nitrate or nitrite ($P<0·05$).

76 days :

Mo_3>Mo_1 with all N sources ($P<0·001$), except ammonium sulphate and ammonium nitrate ($P<0·01$).

101 days :

Mo_3>Mo_1 with most N sources ($P<0·01$), except nitrate plus citrate, ammonium nitrate ($P<0·05$), nitrite (nonsignificant).

Mo_3>Mo_2 with most N sources ($P<0·01$), except ammonium citrate ($P<0·05$) ammonium nitrate (nonsignificant).

There were no significant differences between the Mo_3 and Mo_4 (50 p.p.m.) levels. Effects of molybdenum deficiency decreased with age, but they were consistent irrespective of the type of nitrogen supply.

Concentrations of dehydroascorbic acid produced in different nitrogen or molybdenum treatments rarely differed significantly. There was, however, a tendency both for increased concentrations and greater relative proportions of dehydroascorbic acid to occur at the excess molybdenum level, particularly at 33 and 101 days ($P<0·01$) and for greater proportions at the lowest deficiency level at 76 and 101 days' growth, as shown in Table IV.

Table IV.

Effects of molybdenum supply on mean ratio ascorbic/total ascorbic and dehydroascorbic acids for all nitrogen treatments in 1951.

Mo level	Mo_1	Mo_2	Mo_3	Mo_4	L.S.D.	
	0·00001	0·00005	0·5	50 p.p.m.	$P<0·05$	$P<0·01$
33 days	0·974	0·971	0·933	0·910	0·039	0·052
76 days	0·927	0·959	0·975	0·953	0·032	0·043
101 days	0·893	0·919	0·932	0·847	0·053	0·071

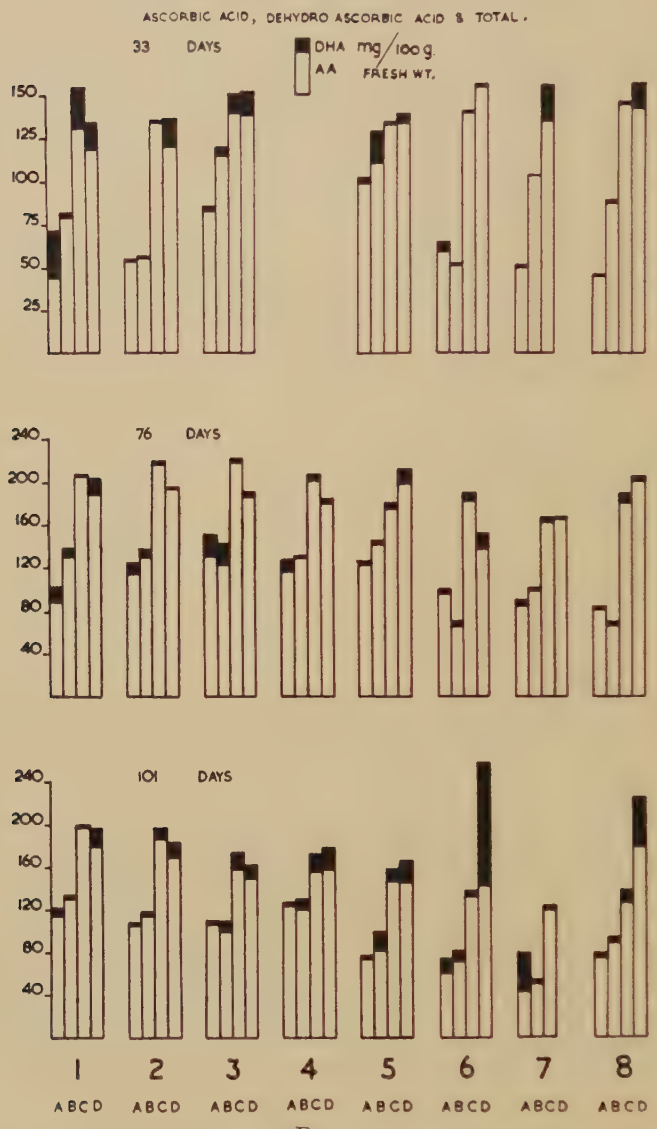

FIG. 2.

Effects of molybdenum level and source of nitrogen on ascorbic (AA) and dehydroascorbic (DHA) acid contents (as mg. per 100 g. fresh wt.) of cauliflower grown in 1951.

Mo level : A, Mo_1 0·000005 p.p.m. ; B, Mo_2 0·00005 p.p.m. ; C, Mo_3 0·5 p.p.m. ; D, Mo_4 50 p.p.m.

N source : 1 Nitrate. 2 Nitrate with added Calcium carbonate.
3 Nitrite. 4 Nitrate with added Citrate.
5 Ammonium sulphate with added Calcium carbonate. 6 Ammonium citrate.
7 Urea. 8 Ammonium nitrate.

Differences for significance at 5 per cent. level :
33 days, AA 29·7 ; 76 days, AA 46·7 ; 101 days, AA 49·4 mg.
AA+DHA 35·7 AA+DHA 44·9 AA+DHA 68·9 m.g.
Note : Mo_4 values for urea missing at 33 and 101 days.

A few estimations of ascorbic acid were also made in 1952 for confirmatory purposes. Results are given in Table V. The effect produced in the presence of ammonium sulphate was consistent with that observed in 1951. The provision of extra sucrose or malate was ineffective in increasing ascorbic acid.

Table V.

Ascorbic acid (mg./100 g. fresh wt.) in leaves of cauliflower plants grown with different nitrogen and molybdenum supplies in 1952.

Nitrogen supply	0·05 p.p.m.	0·00001 p.p.m. Mo
Nitrate	155·2	77·2
Nitrate plus sucrose ..	135·2	75·0
Nitrate plus malate ..	155·2	69·6
Nitrite	175·0	85·3
Ammonium sulphate	187·5	125·2

The molybdenum injection experiments made in 1950 (10) were also repeated in plants given the nitrate treatment for confirmatory purposes, with results shown in Table VI. There was a rapid increase in ascorbic acid as found previously.

Table VI.

Effect on ascorbic acid content (mg./100 g. fresh wt.) of injecting or leaf painting Mo deficient plants with molybdenum.

Time in days	Untreated controls		Deficient plants given Molybdenum		
	+Mo	—Mo	Injected by Mo	Half leaf painted	Unpainted half
0	155·2	77·2			
1	148·6	79·6	73·0	86·4	75·2
3	167·2	69·5	112·5	125·2	85·2
5	159·2	75·4	118·6	130·2	125·2

Sugars.

Total and reducing sugars and sucrose were estimated in leaves at 39, 78 and 145 days' growth. Starch was not detected on these occasions, in agreement with previous observations (5).

The effects of changes in molybdenum supply on sugar concentrations estimated in 1951 decreased with age. Molybdenum deficiency resulted in markedly decreased concentrations of both total and reducing sugars with all nitrogen treatments, except urea, at 39 days. The effect of molybdenum at this time on total and reducing sugars for means of all nitrogen treatments was highly significant : Mo_4 (50 p.p.m.) and Mo_3 (0·5 p.p.m.) > Mo_2 (0·00005 p.p.m.) and Mo_1 (0·000005 p.p.m.) ($P < 0·001$). The trend was similar for sucrose, with lower significance ($P < 0·05$). The greatest differences in sugar concentrations produced by changes in molybdenum supply from 0·000005 or 0·00005 p.p.m. to 0·5 p.p.m. at 39 days occurred in the nitrate, ammonium nitrate and nitrite treatments (Fig. 3). Plants given nitrate or nitrite

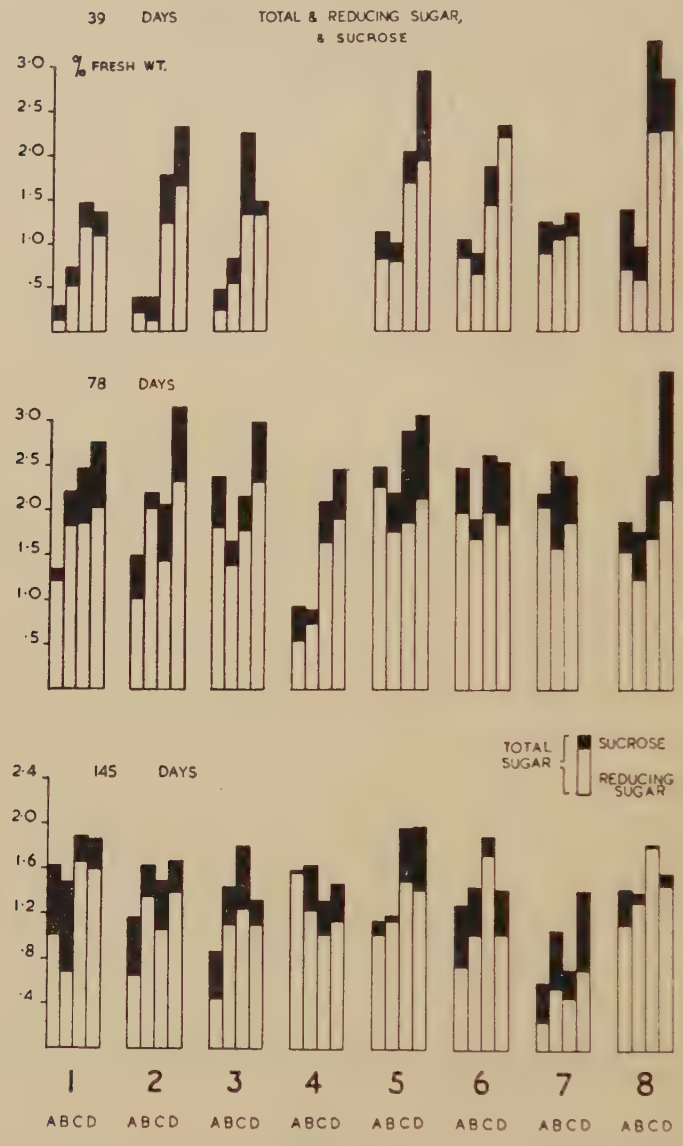

FIG. 3.

Effects of molybdenum level and source of nitrogen on contents of total, reducing sugars and sucrose (as per cent. in fresh wt.) of cauliflower grown in 1951.

Mo level : A, Mo_1 0·000005 p.p.m. ; B, Mo_2 0·00005 p.p.m. ; C, Mo_3 0·5 p.p.m. ; D, Mo_4 50 p.p.m.

N source : 1 Nitrate. 2 Nitrate with added Calcium carbonate.
3 Nitrite. 4 Nitrate with added Citrate.
5 Ammonium sulphate with added Calcium carbonate. 6 Ammonium citrate.
7 Urea. 8 Ammonium nitrate.

Differences for significance at 5 per cent. level :

39 days, Total	1·35 ;	78 days, Total	0·92 ;	145 days, Total	0·78 per cent.
Reducing	0·82	Reducing	0·76	Reducing	0·85 per cent.
Sucrose	0·67	Sucrose	0·73	Sucrose	—

Note : Mo_4 values for urea missing at 39 and 76 days.

usually contained less sugars than those given ammonium compounds at corresponding molybdenum levels. The differences for means of all molybdenum levels were usually significant ($P<0\cdot05$ at 39 days).

Effects of molybdenum supply were less consistent at 78 and 145 days. The general effects of molybdenum for means of all nitrogen treatments were, however, significant as follows :

78 days :

Total sugars : Mo_4 and $Mo_3>Mo_2$ and Mo_1 ($P<0\cdot01$).

Reducing Sugars : $Mo_4>Mo_3$, Mo_2 and Mo_1 ($P<0\cdot05$).

Sucrose : $Mo_4>Mo_2$ and Mo_1 ($P<0\cdot01$) ; $Mo_3>Mo_2$ ($P<0\cdot05$).

145 days :

Total and Reducing Sugars : $Mo_3>Mo_1$ ($P<0\cdot01$).

Plants given nitrate tended to contain less reducing sugars with 0·000005 p.p.m. Mo than those given ammonium treatments at these later times.

Changes in molybdenum levels did not appear to affect sugar content consistently or significantly when urea was given. Increasing the molybdenum supply from 0·5 to 50 p.p.m. did not consistently affect sugar concentrations but, at 78 days, the mean concentrations of total and reducing sugars for all nitrogen sources were increased significantly ($P<0\cdot05$) at the excess molybdenum level.

The statistical significance of effects of changes in molybdenum supplies on concentrations of various sugar fractions shown in Fig. 3 are given in Table VII.

Individual changes in sucrose concentrations were not significant. There were no consistent effects of molybdenum supply on the ratio of sucrose to reducing sugars.

Nitrate content.

Nitrate accumulation in dry matter was examined in both years and results for 1952 are shown in Table II. It is clear that marked nitrate accumulation occurred only in molybdenum deficient plants which had been given nitrate, either alone or with other nitrogen compounds or supplements. Low concentrations of nitrate were apparently present in all plants grown with molybdenum, irrespective of nitrogen treatment, and also in those given non-nitrate sources of nitrogen without molybdenum. Nitrate was either not detected, or was very low in concentration in dry matter of plants grown in 1951 (2) in comparable treatments and accumulated only in molybdenum deficient plants given nitrate in the nutrient solution.

DISCUSSION.

It was concluded from the previous work (3, 4, 5) that molybdenum affects several aspects of growth or metabolism in cauliflower plants given nitrate. These aspects included the occurrence of primary and whiptail symptoms and effects on the contents of ascorbic acid, sugars and chlorophyll. It appeared, however, from the manner in which changes in molybdenum supply affected different aspects of growth or composition that the element might fulfil more than one role, namely that known to mediate nitrate reduction. The evidence for this conclusion is reviewed here in the light of experiments which were designed to distinguish the indirect effects of molybdenum deficiency, due to impaired nitrate reduction, from possibly direct effects of molybdenum on other processes.

TABLE VII.

Statistical significance of effects of changes in molybdenum supply on concentration of sugars produced with different sources of nitrogen shown in Fig. 3.

Age, sugar fractions and effects of molybdenum levels	Significance of molybdenum effect for different nitrogen sources
39 days; Total Sugars:	
$Mo_3 > Mo_1$	Nitrate*, nitrate plus calcium carbonate*, nitrite†, ammonium nitrate†
$Mo_3 > Mo_2$	Nitrate plus calcium carbonate†, nitrite*, ammonium nitrate†
$Mo_4 > Mo_1$ and Mo_2 ..	Nitrate plus calcium carbonate†, ammonium sulphate plus calcium carbonate†, ammonium citrate†, ammonium nitrate†
39 days; Reducing Sugars:	
$Mo_3 > Mo_1$	Nitrate*, nitrate plus calcium carbonate*, nitrite*
$Mo_3 > Mo_2$	Ammonium sulphate plus calcium carbonate*, ammonium nitrate†
$Mo_4 > Mo_1$ and/or Mo_2 ..	Nitrate*, nitrate plus calcium carbonate*, nitrite*, ammonium sulphate*, ammonium citrate†, ammonium nitrate†
78 days; Total Sugars:	
$Mo_3 > Mo_1$ and Mo_2 ..	Nitrate plus citrate*
$Mo_3 > Mo_1$	Nitrate*
$Mo_4 > Mo_2$	Nitrate*, nitrate plus citrate†, nitrite†, ammonium sulphate plus calcium carbonate*, ammonium nitrate†
78 days; Reducing Sugars:	
$Mo_4 > Mo_2$ and/or Mo_1 ..	Nitrate*, nitrate plus calcium carbonate†, nitrate plus citrate†, nitrite†, ammonium nitrate*
145 days; Total Sugars:	
$Mo_3 > Mo_1$ and Mo_2 ..	Ammonium sulphate plus calcium carbonate*
$Mo_3 > Mo_1$	Nitrite*
$Mo_4 > Mo_1$ and Mo_2 ..	Ammonium sulphate plus calcium carbonate*
145 days; Reducing Sugars:	
$Mo_3 > Mo_1$	Nitrite*, ammonium citrate
$Mo_3 > Mo_2$	Nitrate*
$Mo_4 > Mo_1$ and Mo_2 ..	Ammonium sulphate plus calcium carbonate*

* $P < 0{\cdot}05$.
† $P < 0{\cdot}01$.

It is apparent that molybdenum is required by cauliflower for growth as a whole regardless of the source of nitrogen given in these experiments. The occurrence of whiptail symptoms in all nitrogen treatments when molybdenum was omitted shows that this disorder also is not directly or indirectly due to impaired nitrate reduction, contrary to the suggestion (15) that whiptail results from injurious effects of nitrate accumulation. Thus only relatively low concentrations of nitrate occurred in molybdenum deficient and normal plants, grown with the non-nitrate sources of nitrogen, as compared with the much higher concentrations found not only in molybdenum deficient plants given nitrate, but also in normal plants given a relatively very high nitrate supply (5 and unpublished work). The consistent observations (3, 11, present work) that whiptail symptoms are suppressed in molybdenum deficient plants given only nitrate, unless a limited amount of molybdenum is given or unless temporary recovery due to slight contamination occurs, indicate that the appearance of

the disorder actually requires the production of a minimum amount of growth and possibly of organic nitrogen compounds (2). The sharp drop in accumulated nitrate (5) observed at the 0·00005 p.p.m. Mo level, associated with maximum whiptail production (3), is consistent with this view and is also contrary to the idea that whiptail is caused by nitrate injury.

The present experiments also show that the marked decrease in ascorbic acid produced by molybdenum deficiency (4) occurs regardless of the source of nitrogen given. Similar, but relatively less marked, changes in sugar concentrations also occurred at 39 days' growth with all nitrogen treatments used in 1951, except urea.

It was concluded previously (5) that changes in ascorbic acid and sugar concentrations are at least partly independent of one another and possibly indicate distinct effects of some role of molybdenum. The lack of correspondence between changes in these fractions in plants given urea at different ages, or as the result of increasing molybdenum supplies from 0·5 to 50 p.p.m., and the relatively greater sensitivity of ascorbic acid than of sugar concentrations to changes in molybdenum supply, especially at the later samplings, give added support to this conclusion. It is also clear from the present work that the changes in these fractions are not due to limiting effects of inadequate chlorophyll content, in accordance with the conclusions drawn previously (4), or to effects of impaired nitrate reduction.

Thus, in respect of decreased growth, production of whiptail symptoms, decreased ascorbic acid and sugar concentrations, molybdenum deficiency effects appear to be mainly independent of source of nitrogen and of impaired nitrate reduction and indicate the existence of additional roles of the element. The effects of molybdenum deficiency in decreasing the reducing properties of cauliflower petiole tissues to triphenyltetrazolium chloride, regardless of nitrogen source (9), provide further evidence for this general conclusion.

On the other hand, the role of molybdenum in nitrate reduction appears directly to affect the production of the primary symptoms of mottling, wilting and necrosis and to cause great loss of chlorophyll under deficiency conditions, as shown here and in most plants studied (7). The actual accumulation of high concentrations of nitrate under these conditions does not appear to be the only factor responsible, as plants given very high nitrate supplies with adequate molybdenum may also accumulate comparable nitrate concentrations in the leaves (5, and unpublished work) without showing primary symptoms or loss of chlorophyll. Lack of organic nitrogen also does not adequately account for these observations, since many molybdenum deficient plants grown with ammonium nitrate behave in many respects like those grown with nitrate (6, 7, 13), but, unlike the nitrate-fed plants, cauliflower and tomato at least contain high concentrations of several amino acids (2). It must be concluded that primary symptoms and associated loss of chlorophyll are a special response to a molybdenum deficiency—nitrate nutrition interaction and that molybdenum is not otherwise essential for chlorophyll production.

The quantitative molybdenum requirement, as estimated by the relative growth depressions produced by molybdenum deficiency levels, is, however, apparently greatly affected by the source of nitrogen, being greatest with nitrate. In the 1953 and 1954 experiments, plants given ammonium compounds or nitrite and approximately 0·000005 p.p.m. Mo have resembled those given nitrate and 0·00005 p.p.m. Mo as regards the amount of growth and incidence of whiptail. The highly significant molybdenum/nitrogen interaction for yields obtained in 1952 is interpreted as being

the result of a greater requirement for growth in the presence of nitrate than of non-nitrate nitrogen sources. The significance of these apparent differences in molybdenum requirements is not clear at present, as many factors are probably involved. Thus it appears that requirements might be increased further by inclusion of citrate, malate or sucrose into the nutrient medium without decreasing molybdenum uptake.

The effects of excess of molybdenum merit a brief comment. The production of blue or golden pigments has been described (3, 14). It is interesting to note that the distribution of blue pigment in the nitrite plants given 50 p.p.m. Mo corresponded to that of a probable anthocyanin in leaves of the plants given 0·5 p.p.m. Mo. Different nitrogen treatments possibly determined whether blue or golden pigments predominated. The blue pigment was intense with urea as the nitrogen source and the appearance of a blue pigment also in the sand raises the question whether the so-called "molybdenum-anthocyanin" blue pigment is a real entity, or whether the anthocyanin is merely a suitable reagent for the production of molybdenum blue *in vivo*. The most striking result was the marked interaction between nitrogen source and the injury from molybdenum excess, which probably arose from the differences in molybdenum uptake at the 50 p.p.m. level. The effects of molybdenum excess in sometimes producing greater sugar concentrations than those found at the 0·5 p.p.m. level are not regarded as indicative of the physiological role of the element.

SUMMARY.

1. Cauliflower plants, variety Majestic, were grown in sand culture with nitrogen given either as nitrate, or as nitrite, ammonium compounds, or combinations of these, or as urea or as glutamic acid at deficiency, normal and, in some treatments, at excess levels of molybdenum.

2. Primary molybdenum deficiency symptoms of mottling, wilting and scorching occurred consistently but only in the presence of nitrate-containing treatments. They are regarded as the result of nitrate nutrition-molybdenum deficiency interaction as contrasted with the general relationship of whiptail to molybdenum deficiency.

3. Whiptail symptoms indicating molybdenum deficiency were produced at low molybdenum levels irrespective of the nitrogen source. They are regarded as evidence of some role of molybdenum additional to that in nitrate reduction.

4. Yields were decreased by molybdenum deficiency irrespective of nitrogen source.

5. Molybdenum requirement, as judged by growth depression or incidence of whiptail and other symptoms under deficiency conditions, was greatest with nitrate or ammonium nitrate, and least with ammonium and nitrite compounds. Addition of citrate or malate apparently accentuated molybdenum deficiency symptoms with nitrate or nitrite, but not with urea or ammonium compounds.

6. Effects of molybdenum excess differed with different sources of nitrogen. Toxic effects were slight with nitrate, severe with ammonium sulphate or urea and intermediate with nitrite and ammonium nitrate.

7. Molybdenum content in leaves was not appreciably affected by nitrogen supply or by addition of citrate or malate under deficiency or normal conditions. At

PLATE I.

Fig. 4.

Examples of whiptail in cauliflower produced in plants given different sources of nitrogen in molybdenum deficient sand cultures.

(*a*) Ammonium sulphate at approximately 0·00001 p.p.m. Mo.
(*b*) Ammonium nitrate at approximately 0·00001 p.p.m. Mo.
(*c*) Glutamic acid at approximately 0·00001 p.p.m. Mo.
(*d*) Ammonium nitrite at approximately 0·000005 p.p.m. Mo.
(*e*) Urea at approximately 0·00001 p.p.m. Mo.
(*f*) Nitrite at approximately 0·000005 p.p.m. Mo.

[*face p. 178*

the excess level, molybdenum accumulation was greater with urea or ammonium sulphate.

8. Chlorophyll content was decreased by molybdenum deficiency when nitrate was given, regardless of the presence of other nitrogen compounds. In the absence of nitrate, chlorophyll content was not significantly affected by molybdenum supply.

9. Ascorbic acid content was decreased by molybdenum deficiency at 33, 76, and 101 days' age in plants given nitrate, nitrite, ammonium compounds or urea. The ratio dehydroascorbic/ascorbic acid was usually slightly increased at deficiency and excess molybdenum levels as found previously.

10. Total and reducing sugars were decreased in leaves by molybdenum deficiency with nitrate, nitrite or ammonium nitrogen sources, but not with urea at 39 days' age. Changes were most marked in young plants and were only observed in more limited instances at later samplings.

11. Marked nitrate accumulation was detected in molybdenum deficient plants only when given nitrate. In plants given molybdenum or the other sources of nitrogen, the nitrate content was uniformly low.

12. The effects of molybdenum deficiency on growth and whiptail production and on ascorbic acid and sugar contents were independent of those on chlorophyll content or nitrate accumulation. The effects of molybdenum on ascorbic acid content were apparently independent of those on sugar content.

13. It is concluded that molybdenum is an essential element for cauliflower, regardless of whether nitrogen is given as nitrate or as nitrite, ammonium compounds, urea or glutamic acid, and that it has a multiple role in the metabolism of cauliflower in addition to that in nitrate reduction.

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THE EFFECTS OF ALDRIN, DIELDRIN, ISODRIN, ENDRIN AND DDT ON MITOSIS IN ROOTS OF THE ONION *(ALLIUM CEPA* L.*)*

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SYNOPSIS.

Experiments were carried out with aldrin, dieldrin, isodrin, endrin and DDT to ascertain whether, employed as insecticides, these compounds had any effect on cell division in onion root-tips. The doses used were equal to, or greatly in excess of, the recommended rates of application. By germinating onion seeds in direct contact with the insecticide under investigation, it has been shown that none of the compounds had any effects on germination or growth rate. High doses of aldrin, dieldrin or isodrin had a slight toxic effect on the resting cell, which, with aldrin, was also caused by the re-crystallized compound. Aldrin and dieldrin had no effect on the dividing cell. Isodrin and endrin caused slight stickiness between chromatids in anaphase, which was insufficient to prevent the completion of division in the cell, and DDT caused a delay in the early stages of prophase and a shortening of chromosomes in metaphase.

Pot experiments, with high doses of each compound, showed that germination and growth rate were unaffected by any of the compounds. Resting cells were not affected and dividing cells were affected only by slight stickiness in anaphase, except with DDT, which also caused a slight delay in metaphase.

Attention has been drawn to the advantages of these compounds over *gamma*-BHC, chlordane and toxaphene as insecticides, from the point of view of causing less damage to the plant.

Introduction.

The effects of *gamma*-BHC (hexachloro*cyclo*hexane), chlordane and toxaphene on mitosis in roots of the onion (*Allium cepa* L.) have been described in previous papers (Scholes, 1953 and 1955). This paper records the results of similar experiments with aldrin, dieldrin, isodrin, endrin and DDT. Aldrin and dieldrin are coming into use on a much larger scale for the control of insects of wide economic importance such as wireworms, flea beetle larvae, cabbage and onion maggots, cutworms, and many others. Isodrin and endrin are newer insecticides, but show great promise in their versatility of action; control of such completely different insects as aphids and cut-worms has been obtained by their use. All four compounds have the advantage of being very stable chemically, even in the presence of alkalies. The properties of DDT are too well known to need further mention.

In general, all these compounds are considered to be non-phytotoxic, the only reports of damage to crops being due to extreme doses. Tiedjens (1952) reported a reduction in the germination of lettuce seed and a delay in the emergence of bean seeds at an extreme dose of 100 lb. aldrin per acre. Cox and Lilly (1952) also reported poor development of a number of field crops with high doses of aldrin, but good growth of crops treated with lower doses or with dieldrin at rates as high as 128 lb. per acre. Julius Hyman and Company, in their 1951 progress reports of the development of isodrin and endrin, reported slight " burning " of corn buds and cucumber foliage when plants were sprayed with these compounds, but this had not been further investigated. Reports of phytotoxic effects of DDT have been summarized by West and Campbell (1950).

It seemed useful, therefore, to investigate the effects of all these compounds on the onion root when applied at rates recommended for use in the field.

THE INSECTICIDES.

In these experiments aldrin, re-crystallized aldrin (m.p. 99·5°-100·5°C.), dieldrin, isodrin, endrin and *pp'*-DDT (m.p. 108°C.) were used as dusts on gypsum. The composition of the dusts, the recommended rates of application and doses used are shown in Table I. Re-crystallized aldrin had been obtained from aldrin by crystallizing from acetone, passing through a column of alumina and finally re-crystallizing from benzene. Aldrin, dieldrin and DDT were mixed with gypsum by the "butter churn" method. Isodrin and endrin were dissolved in acetone, poured on to the gypsum, the acetone allowed to evaporate and the mixture turned in a " butter churn ".

TABLE I.

Composition, recommended application and doses of insecticides used in Petri dish experiments, showing the equivalent doses in lb. per acre.

gm./Petri dish compound on gypsum	lb./acre actual compound	lb./acre compound on gypsum	Per cent. compound in dust	Recommended rate of application actual compound in lb./acre
0·023 aldrin	0·75	30	2·5	0·25–0·75
0·18 aldrin	6	240		
0·18 re-crystallized aldrin ..	6	240	2·5	
0·298 dieldrin	6	400	1·5	0·25–0·5
0·03 isodrin	0·4	40	1·0	0·1 – 0·4
0·15 isodrin	2	200		
0·03 endrin	0·4	40	1·0	0·1 – 0·4
0·15 endrin	2	200		
0·109 DDT	4·4	145	3·0	4·4 –11·0
0·436 DDT	17·6	580		

PETRI DISH EXPERIMENTS.

Seeds of onion, variety Ailsa Craig, were germinated in Petri dishes under the same conditions as those described in a previous paper (Scholes, 1953). They were directly in contact with the insecticide under investigation. The temperature, recorded by a maximum and minimum thermometer, was allowed to vary daily between 56° F. and 80° F., for controls and treated seedlings, to simulate conditions in the field. Fixations were carried out at similar times each morning, to allow for the effects of this variation on the mitotic index. The pH of the wet filter paper was measured at the end of each experiment by Johnson's Comparator Test Paper No. 6883 and was of the order of 7·4.

Root-tips were fixed in acetic-alcohol 48 hours after germination. The control roots attained a length of 1 cm. in this time, and this was taken as the standard of normal growth. Feulgen root-squashes were prepared. The mitotic index and mitotic formula were determined for six control roots and six roots in each experimental group by counting 2,500–3,500 cells in each root. The results are shown in Table II.

None of the compounds under investigation had any effect on germination or growth rate of onion seedlings under the conditions of these experiments.

Cytological effects of aldrin and dieldrin.

Aldrin, re-crystallized aldrin or dieldrin had no effect on the dividing cell, but there was evidence of a toxic effect on the resting cell, affected nuclei appearing small, deeply stained and moribund. In determining the mitotic index these cells were not counted as viable cells, and this accounts for the significant increase in the mitotic index caused by the re-crystallized aldrin or dieldrin. The effect was evident in roots treated with aldrin but was insufficient to cause significant changes. Cox and Lilly (1952), using honey sorghum as test material, obtained better germination and growth with re-crystallized aldrin than with aldrin equivalent containing 17·9 per cent. related compounds, both applied at the same levels ; they concluded that the associated materials were more phytotoxic than the aldrin itself. These experiments with onion seedlings confirm that aldrin and also dieldrin have some phytotoxic effect which, with aldrin, was slightly increased by increasing the concentration of the actual aldrin.

Cytological effects of isodrin and endrin.

Isodrin and endrin had very little effect on either the resting or dividing cell. There was evidence that each compound caused a slight stickiness in anaphase which delayed the separation of the chromatids, but was insufficient to prevent it. With the higher dose of isodrin, there was also evidence of a slight toxic effect on the resting cell similar to that caused by aldrin.

Cytological effects of DDT.

In these experiments both doses of DDT caused significant increases in the number of prophases seen. The majority of these prophases were in the early and middle stages, indicating delay at an earlier stage than that caused by chlordane and toxaphene (Scholes, 1955). The chromosomes in metaphase were orientated on the spindle but were severely shortened. Vaarama (1947) made a similar observation and regarded this shortening as the first approach to polyploidy, suggesting that the poor solubility of DDT in water accounted for the absence of other c-mitotic effects.

POT EXPERIMENTS.

The soil used in these experiments was garden loam, partially sterilized by steaming at 180° F. and watered with rain water. The insecticide was spread on the surface layer, mixed with it to simulate broadcasting in the field, and the seed covered with $\frac{1}{8}$ in. of soil. The doses used are shown in Table III. The temperature during these experiments varied between 52° F. and 74° F. Thirty seeds were sown in each group. Seedlings were raised 10 days after sowing and six root-tips from each group taken from roots varying in length from 1·5–2·5 cm., were fixed and examined cytologically.

Table IV shows the percentage germination and growth rate for onion seedlings treated with heavy doses of the insecticides. The variations shown are not significant ; they are within the normal variation for untreated seedlings from the sample of seed used.

TABLE II.

Comparison of mitotic index and mitotic formula for controls and roots treated with aldrin, re-crystallized aldrin, dieldrin, isodrin, endrin or DDT.

Treatment. Dose in gm. compound on gypsum	Mean % dividing cells	Mean % prophases	Mean % metaphases	Mean % anaphases	Mean % telophases
Controls	15·3	46·4	9·5	2·1	41·9
Standard error of control mean	0·27	0·87	0·41	0·26	0·84
0·023 aldrin	15·0	48·6	9·7	2·8	39·0
0·18 aldrin	15·9	46·8	9·0	2·2	41·9
0·18 re-crystallized aldrin	17·7	46·0	9·1	2·3	42·6
Significant difference from controls (P=0·05) ..	1·14	3·10	1·61	0·79	2·68
0·298 dieldrin	17·6	47·6	8·7	2·1	41·7
Significant difference from controls (P=0·05) ..	2·07	3·43	1·79	0·83	3·73
0·03 isodrin	16·5	48·5	8·5	3·5	39·4
0·15 isodrin	17·2	45·0	8·6	2·9	43·4
Significant difference from controls (P=0·05) ..	1·25	2·87	1·95	0·98	2·95
0·03 endrin	16·1	48·5	8·7	3·8	38·9
0·15 endrin	16·7	44·8	9·8	1·9	43·4
Significant difference from controls (P=0·05) ..	1·72	3·19	1·78	0·97	3·60
0·109 DDT	17·0	53·1	8·3	2·3	36·3
0·436 DDT	16·0	54·6	7·8	2·1	35·5
Significant difference from controls (P=0·05) ..	2·06	3·92	1·39	0·73	3·96

TABLE III.

Doses of insecticides used in pot experiments.

Treatment. gm./pot compound on gypsum. (Surface area 0·013 sq. yd.)	lb./acre compound on gypsum	lb./acre actual compound
0·29 aldrin	240	6
0·49 dieldrin	400	6
0·70 DDT	580	17·4
0·24 isodrin	200	2
0·24 endrin	200	2

TABLE IV.

Percentage germination and lengths of roots for onion seedlings treated with aldrin, dieldrin, isodrin, endrin or DDT.

Treatment	Percentage germination	Number of roots in length groups		
		2–4 cm.	1–2 cm.	Less than 1 cm.
Aldrin	86·6	14	8	4
Dieldrin ..	70·0	9	4	8
Isodrin	80·0	5	14	5
Endrin	70·0	8	6	7
DDT	60·0	10	6	2

Cytological effects.

The mitotic formula was determined for roots treated with DDT ; but in all other treatments, 300–400 dividing cells were examined in each root for chromosome breakage and incomplete separation of chromatids in anaphase. There was no evidence of a toxic effect on the resting cell. Table V shows that the dose of DDT used caused significant delays in metaphase and anaphase but there was no evidence

TABLE V.

Comparison of mitotic formula for controls and roots treated with DDT in soil.

Treatment	Mean % prophases	Mean % metaphases	Mean % anaphases	Mean % telophases
Controls	46·4	9·5	2·1	41·9
17·4 lb./ acre DDT ..	46·8	12·4	4·3	36·7
Significant difference from controls (P=0·05) ..	3·95	2·04	1·36	5·67

of delay in prophase, nor shortening of the chromosomes in metaphase. Table VI shows that all the compounds under investigation brought about an increase in the number of anaphases showing incomplete separation of chromatids, which is probably due to stickiness induced by the treatment and may account for the delay in metaphase caused by DDT. This was not, however, sufficient to affect the growth rates.

The numbers of cells with visible chromosome breaks in the control and treated roots are interesting, since the Petri dish controls show the highest number. Levan and Lofty (1950) observed chromosome breaks in roots of *Vicia faba* seedlings which could not be correlated with the chemical treatment under investigation. They found that the frequency of occurrence of the fragments decreased with increasing age of the seedlings and that it was less in young seedlings grown in soil than in those grown in Petri dishes after soaking the seed in water for 24 hours. The present experiments show a similar result with *Allium cepa*. Harrison and McLeish (1954) reported chromosomal aberrations in *Crepis* and *Allium* seedlings raised from old seed. They found, however, that ageing alone was not responsible for the changes ; the effects of age could be overcome by storage conditions.

TABLE VI.

Comparison of the percentage of breaks and anaphase bridges for control roots grown in Petri dishes or untreated soil and roots grown in soil with doses of aldrin, dieldrin, isodrin, endrin or DDT.

Treatment. gm./pot compound on gypsum	No. of metaphases + anaphases examined	% cells with visible chromosome breaks	% anaphases showing incomplete separation of chromatids
Petri dish controls ..	326	5·8	25·0
Pot controls	263	3·1	27·0
0·29 aldrin	232	1·3	34·6
0·49 dieldrin	256	0·8	48·5
0·24 isodrin	241	2·9	42·5
0·24 endrin	288	1·1	44·0
0·70 DDT	320	0·3	38·0

DISCUSSION.

The Petri dish experiments show that onion seedlings can be grown in direct contact with heavy doses of aldrin, dieldrin, isodrin and endrin with little effect on the dividing cell. DDT had more effect than these compounds, but less than *gamma*-BHC, chlordane or toxaphene. Heavy doses were used in the pot experiments, but the effects were slight, DDT again having the greatest effect. There was no direct evidence of factors that would cause genetical changes.

The cytological effects of the chlorinated hydrocarbons tested in this series of experiments appear at first sight to follow the degree of dehydrochlorination of the compounds when in contact with dilute alkalies. The *beta* isomer of BHC liberates less hydrogen chloride than the *alpha*, *gamma* or *delta* isomers or DDT and has less effect on mitosis, whilst aldrin, dieldrin, isodrin and endrin do not liberate hydrogen chloride and have no effect on mitosis. Lord (1948) found that some decomposition of DDT took place in a buffer at a pH of 7·1, and in general the speed of the reaction increased with increasing pH. At a pH of the order of 7·4 in the Petri dish experiments, the compounds that can dehydrochlorinate would be likely to do so. Albert (1951) considered that the amount of hydrogen chloride liberated by DDT would be too small to affect the presumably well-buffered tissues of insects, and this would appear to apply to plants also. He regarded the chlorinated hydrocarbons as structurally non-specific agents, acting simply as foreign bodies in the cell and in accordance with Ferguson's Principle. It is, however, impossible to compare the solubilities of these compounds in distilled water. Slade (1945) stated that the solubility of the *beta* isomer of BHC was consistently low in comparison with that of the other isomers but there are no comparative figures available for the group of compounds.

Aldrin, dieldrin, isodrin and endrin are likely to become increasingly important in the control of certain insects. These experiments have shown that they are less phytotoxic and less likely to cause cytological derangements than *gamma*-BHC, chlordane, toxaphene and DDT (though here the difference is smaller), even when applied in amounts greatly in excess of those recommended.

SUMMARY.

1. Onion seeds have been germinated in contact with a 2½ per cent. mixture of aldrin or re-crystallized aldrin, a 1½ per cent. mixture of dieldrin, a 1 per cent. mixture of isodrin or endrin and a 3 per cent. mixture of DDT on gypsum.

2. The doses used, when calculated in pounds per acre of the dusts, were equivalent to 30 and 240 aldrin, 400 dieldrin, 40 and 200 isodrin or endrin, 145 and 580 DDT.

3. The compounds had no effect on germination or growth rate of onion seedlings.

4. Aldrin, re-crystallized aldrin, dieldrin and the higher dose of isodrin had a slight toxic effect on the resting cell.

5. Aldrin, re-crystallized aldrin and dieldrin had no effect on the dividing cell. Isodrin and endrin caused only slight stickiness in anaphase. DDT caused a delay in the early stages of prophase and shortening of the chromosomes in metaphase.

6. In pot experiments, with the higher dose for each compound, germination and growth rate were not affected. DDT caused a slight delay in anaphase.

7. The numbers of spontaneous chromosome breaks in onion seedlings germinated in Petri dishes and in soil have been compared.

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SEASONAL CHANGES IN THE MAJOR NUTRIENTS OF BLACK CURRANT LEAVES

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During the past 30 years increasing attention has been paid to plant analysis as a guide to the nutritional requirements of plants, as distinct from the original conception of plant analysis as an index of soil fertility. Although the nutritional requirements of certain crops and tree fruits have been investigated by this method, soft fruits have received less attention.

Before the results of plant analysis can be interpreted successfully certain basic information is required. This includes a knowledge of the seasonal changes in nutrient status within the various plant organs, of the most appropriate organ for analysis, and of the most suitable time of sampling. Having decided on the last two factors, which are not necessarily the same for all nutrients in the same crop, the next step is to establish nutrient levels within the plant organ associated with deficiency symptoms, optimum growth and luxury consumption. Many complications arise due to interactions between plant nutrients and to other growth factors. These have been reviewed and discussed at length by Goodall and Gregory (3), Lundegårdh (4), Ulrich (5) and others.

The aims of the present investigation were : (i) to obtain information on the nutrient changes in black currant leaves with respect to season and position on the shoot, thereby making it possible to reduce leaf sampling errors in later experiments, (ii) to determine at what stage in leaf development changes in nutrient levels were at a minimum, so that differences due to manurial treatments could be compared most accurately and (iii) to provide data on the seasonal concentration of major nutrients in leaves from bushes growing under good conditions with respect to management, manurial treatment and moisture supply.

Previous studies on black currant leaf samples, taken from the mid-third region of current-season growths prior to fruit maturity (early July), indicated that this method of sampling was satisfactory for comparing treatment effects with respect to nitrogen. Furthermore the total leaf nitrogen at this stage, all other nutrients being present in adequate amounts, was positively correlated with the yield of fruit (1, 2).

The present investigation was undertaken before starting on a comprehensive study of the relationship between leaf nutrient status, plant growth and yield of fruit in black currants, using material and data from large-scale NPK factorial field experiments.

Experimental.

History of bushes.

The bushes (variety Mendip Cross) were planted in 1944 on a heavy sand overlying Keuper Marl. On analysis the top 9 in. of soil contained 0·026 per cent. P_2O_5 and 0·016 per cent. K_2O soluble in 1 per cent. citric acid, and 1·57 per cent. organic carbon.

Each year in March the plot received a surface dressing at the rate of 6 cwt. of nitro-chalk and 2 cwt. of potassium chloride per acre, and amounts of superphosphate varying from 3–6 cwt. per acre. The bushes grew well, cropped heavily and showed no deficiency symptoms, although the nitrogen status was probably sub-optimal at the time samples were taken.

Leaf sampling.

Sampling was started on 5th May, and repeated at weekly intervals till 17th June and finally on 3rd July, 40 extension shoots (per sample) of approximately equal length being chosen at random (not more than two per bush) and cut off where the new wood joined the fruiting wood. The samples were always taken in the early afternoon. Within an hour of picking, the leaves plus petioles were removed in ascending order from the shoots and bulked according to their position on them. Thus each composite sample consisted of 40 leaves. The unopened terminal clusters of leaves were combined and are referred to as " tip leaves " in the text figures. The dry matter obtained from these tip leaves in the early samples was insufficient for a complete analysis, hence the missing values for P, K, Ca and Mg in Figs. 2–5.

Analytical methods.

The leaves were dried at 100° C., ground, and sub-samples analysed for total N, P, K, Ca and Mg. Nitrogen was determined on separate samples, by a procedure which includes a semi-micro digestion and micro-distillation, the ammonia being trapped in 0·5 per cent. boric acid and back titrated with N/70 H_2SO_4. The other elements were determined on the same sub-sample, after oxidation of organic matter with mixed nitric and perchloric acids. Phosphorus was determined colorimetrically by the phosphovanadate method and potassium and calcium by flame photometry. Calcium was first separated from phosphate by precipitation as the oxalate and then taken up in a known volume of dilute nitric acid. Magnesium was determined by a titan yellow colorimetric method. The method for magnesium was not entirely satisfactory and less reliance can be placed on these values than on those for the other constituents. Analytical errors for N, P, K and Ca were about 2 per cent.

Results.

Nitrogen.

Leaf nitrogen decreases with age, both positional and seasonal, until early June (Fig. 1). At this stage differences due to position are at a minimum and the levels remain fairly constant over the next 2–3 weeks, i.e. up to fruit maturity.

Phosphorus.

The seasonal trend in the leaf phosphorus status (Fig. 2) follows the same general pattern as for nitrogen until early June, after which time the concentration of phosphorus in the older leaves remains fairly constant, whereas that of the later formed leaves (8–12) continues to fall.

The positional variation differs from that of nitrogen. Starting from the base the leaf phosphorus falls steadily over the first third of the shoot, remains fairly constant over the middle third region and then rises again towards the tip leaves until early June, after which time there is little difference between leaves from the upper two-thirds of the shoot (with the exception of the " tip leaves ").

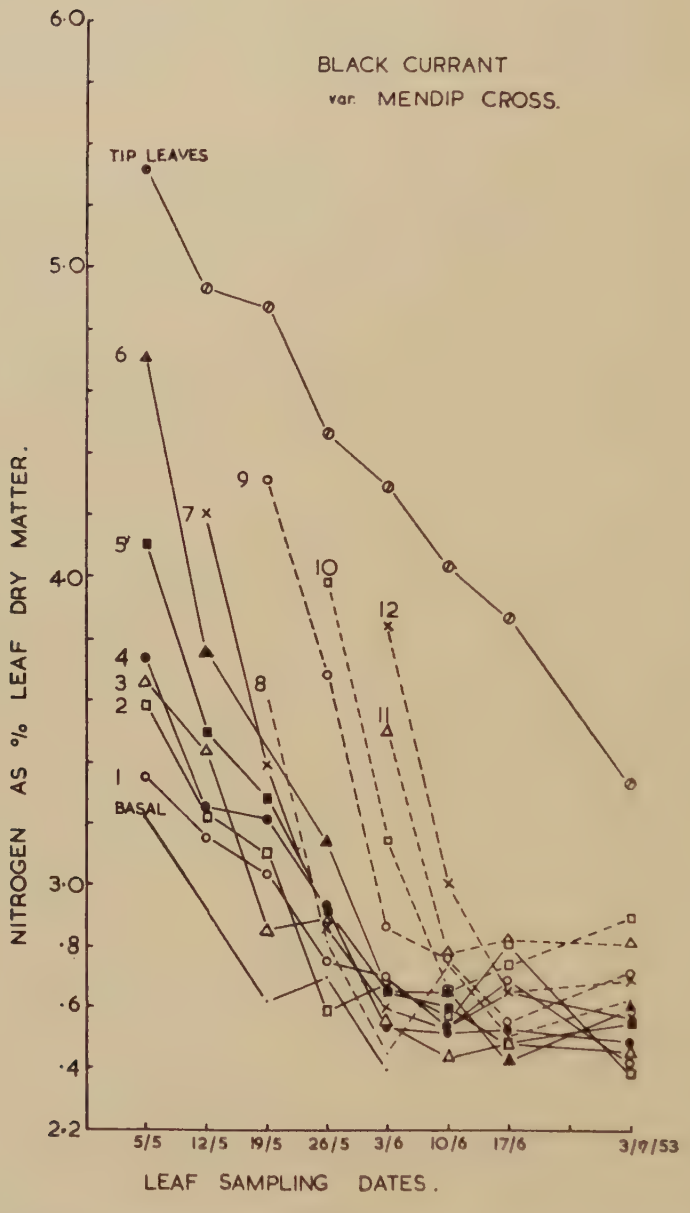

FIG. 1.

Positional and seasonal variation in the nitrogen content of black currant leaves from current-season's extension shoots. Numbers refer to leaf position relative to the basal leaf.

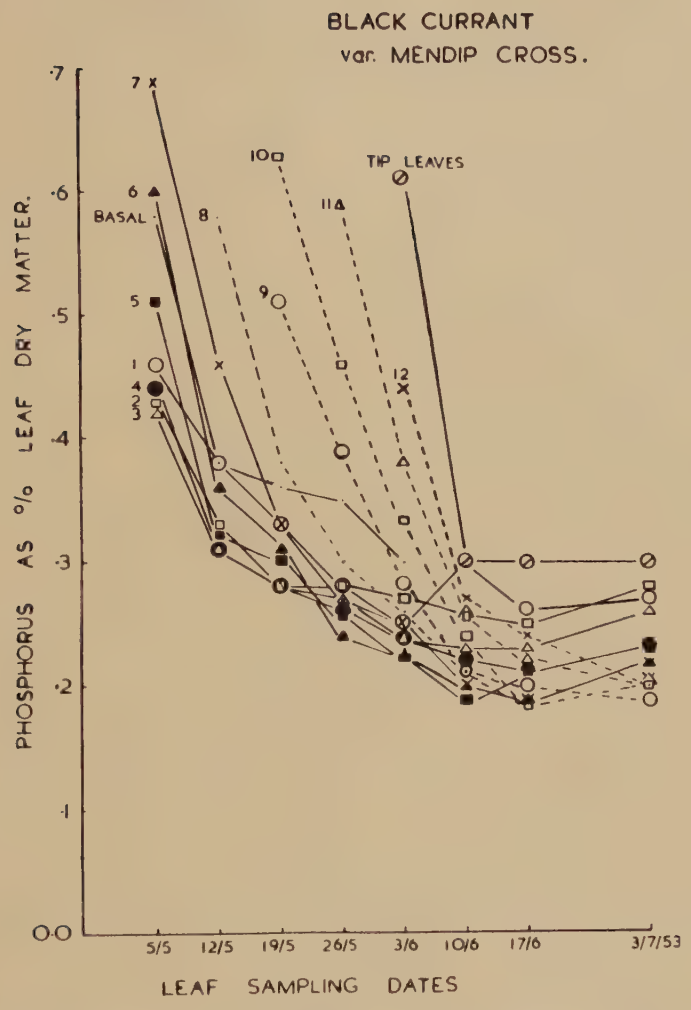

Fig. 2.

Positional and seasonal variation in the phosphorus content of black currant leaves from current-season's extension shoots. Numbers refer to leaf position relative to basal leaf.

Potassium.

With the exception of the basal and first leaf in the early samples, the potassium concentration decreases from base tip and with season, the seasonal decrease being greatest in the first formed leaves (Fig. 3). Positional differences are least towards the end of June and changes in concentration during the two weeks prior to fruit maturity are only slight.

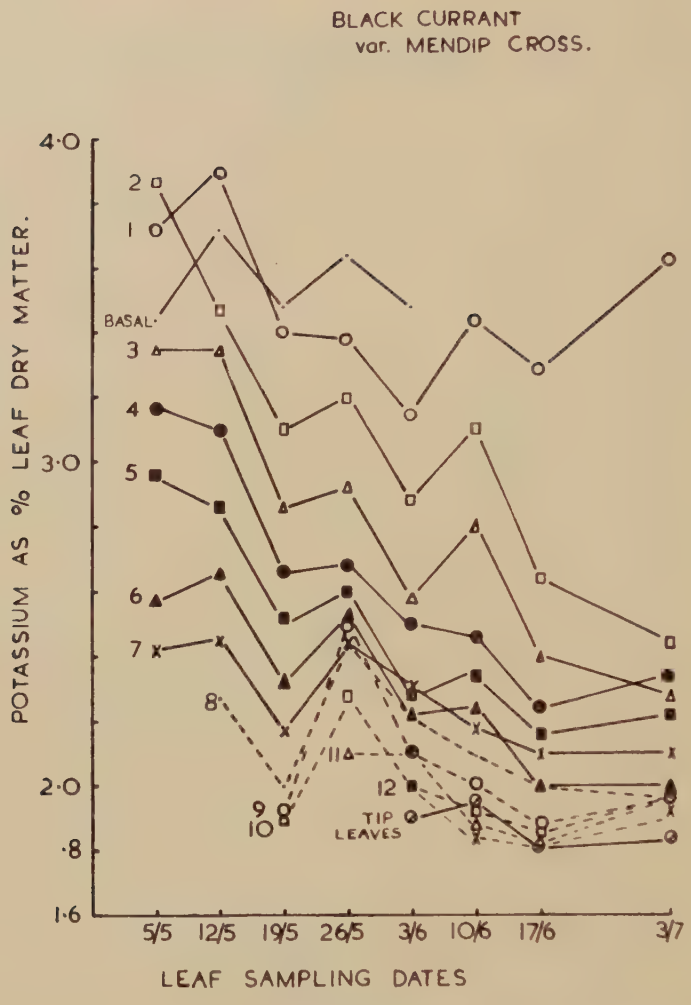

Fig. 3.

Positional and seasonal variation in the potassium content of black currant leaves from current-season's extension shoots. Numbers refer to leaf position relative to the basal leaf.

Calcium.

The calcium concentration decreases rapidly from base to tip and, with the exception of the basal leaf, increases almost linearly with season (Fig. 4). Unlike the previous nutrients the positional differences remain much the same throughout the season.

Magnesium.

The magnesium concentration decreases from base to tip and increases with season, with least variation in the middle third region (Fig. 5). Seasonal fluctuations in the first formed and young leaves are fairly large.

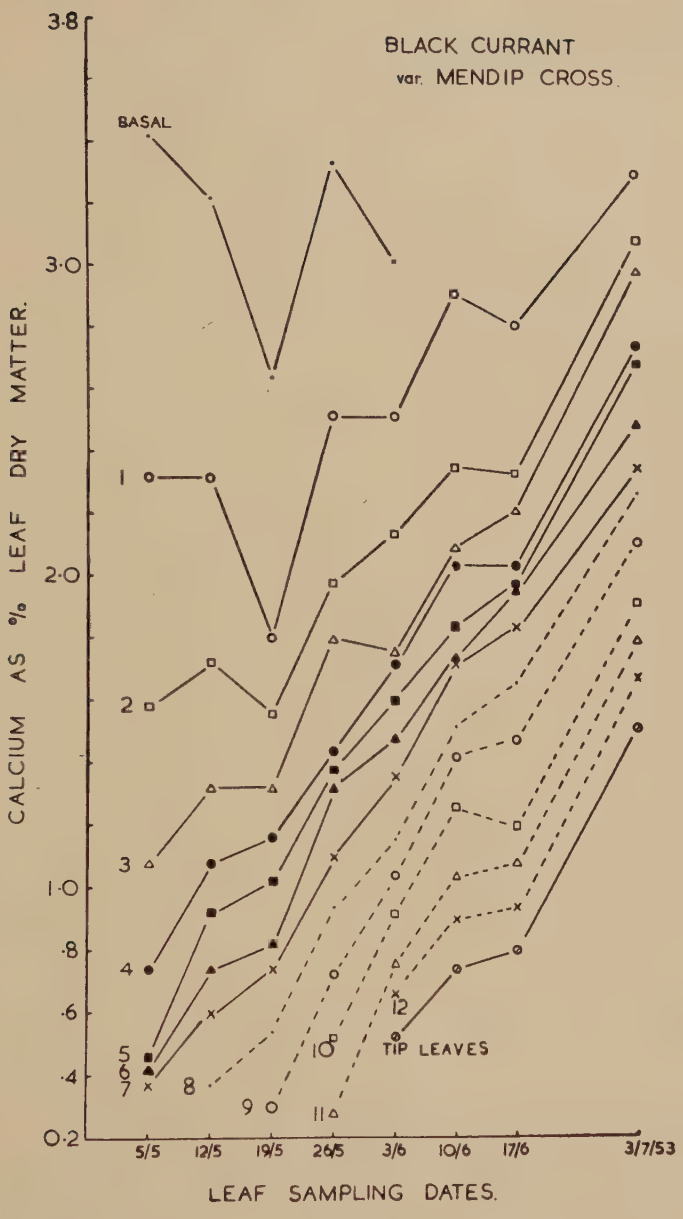

FIG. 4.

Positional and seasonal variation in the calcium content of black currant leaves from current-season's extension shoots. Numbers refer to leaf position relative to the basal leaf.

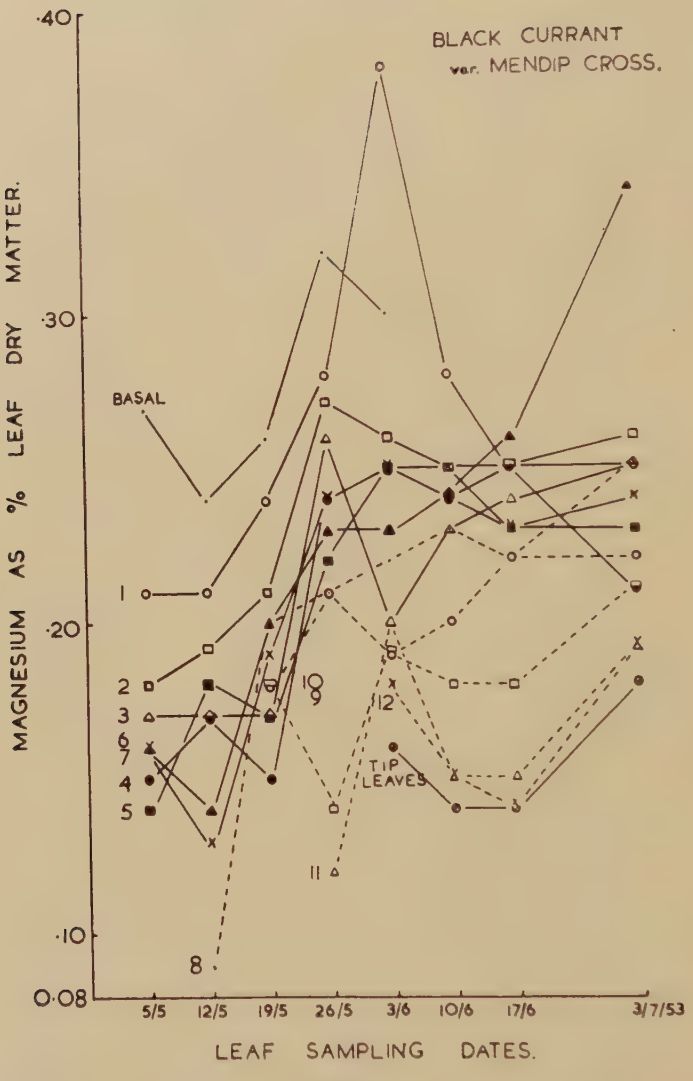

FIG. 5.

Positional and seasonal variation in the magnesium content of black currant leaves from current-season's extension shoots. Numbers refer to leaf position relative to the basal leaf.

DISCUSSION.

From the results it is clear that changes in the major nutrient concentration of black currant leaves occur with position and season. For nitrogen and phosphorus the differences due to position are at a minimum two to three weeks prior to fruit maturity, and furthermore the concentration of these two nutrients in leaves from the middle third region of current growth during this period is fairly constant. Therefore by taking leaf samples during this period slight variations in position, which are inevitable in rapid random sampling when one is dealing with large numbers of samples on the same day, would cause no appreciable error. It is thought also that, since the levels of nitrogen and phosphorus during this period remain fairly constant, this would be the most favourable period for collecting samples for comparative and diagnostic purposes. Previous experiments (1) showed that leaves from the middle third region of current-season shoots, taken prior to fruit maturity, were suitable for comparing the effects of varying levels of nitrogenous manures.

The picture is not quite so clear for potassium. Although positional and seasonal variations were least prior to fruit maturity, it should be borne in mind that the potassium level in all leaves was high, and that seasonal changes might be quite different under less favourable nutritional conditions. Until further information is available it is suggested that the same sampling method be adopted for potassium and magnesium as for nitrogen and phosphorus.

The positional differences for calcium remain fairly constant and, as the seasonal differences over the period studied are almost linear, no preferential time of sampling is indicated.

Leaf sampling was discontinued prior to fruit picking because leaves may be badly damaged by the pickers, and leaf spot (*Pseudopeziza ribis*) may also cause severe defoliation about this time, thus making impossible a complete sampling of all leaves from the current shoots.

SUMMARY.

1. The positional and seasonal changes in the N, P, K, Ca and Mg contents of current growths from black currant, variety Mendip Cross, are described and discussed in relation to leaf sampling for comparative and diagnostic purposes.

2. Leaf nitrogen decreases with position and season until early June, when positional differences are at a minimum. Nitrogen levels remain fairly constant for the next three weeks.

3. The seasonal trend in the leaf phosphorus content is similar to that of nitrogen until early June, after which time the concentration in the older leaves remains fairly constant, whilst that of the later formed leaves continues to fall. Positional variation differs from that of nitrogen.

4. The potassium concentration decreases with age of leaf in respect of position and season.

5. The leaf calcium concentration decreases from base to tip and increases almost linearly with season.

6. The leaf magnesium concentration decreases from base to tip and increases slightly with season, with least variation in the middle third region of the shoot.

7. It is suggested that leaf samples taken from the middle third region of current shoots just prior to fruit maturity are suitable for comparative and diagnostic purposes in respect of N, P, K and Mg.

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I am indebted to Miss B. Hood who was responsible for most of the analytical work, and for drawing the graphs.

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BLACK CURRANT LEAF SPOT:

II. LABORATORY TESTS OF FUNGICIDES FOR THE PREVENTION OF SPORING OF *PSEUDOPEZIZA RIBIS* ON OVERWINTERED LEAVES

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THE presence of the perfect stage of *Pseudopeziza ribis* on overwintered fallen black currant leaves at Long Ashton has already been reported (Corke, 1954). The same paper gave the results of preliminary experiments made in 1953 to examine the effects on leaf spot incidence of three methods of combating the disease during the winter and spring. Significant reduction in leaf spot attack resulted from spraying the stools and the surrounding ground in February with 0·1 per cent. dinitro-*ortho*-cresol, also from the removal of dead leaves by raking, and from the application of a 1·5 per cent. lime-sulphur spray to the bushes in early April. Any two of these treatments together proved more effective than a single treatment, while a combination of all three gave the greatest reduction in leaf spot infection in the ensuing summer.

It appeared, therefore, that more detailed trials were warranted on the choice and concentrations of fungicides to be used on overwintered black currant leaves, with the object of reducing the inoculum available in the spring and thereby delaying the onset of leaf spot. The materials selected for the trials described below were mainly those that had shown value as eradicants when used against the overwintering stages of apple canker (*Nectria galligena*) (Byrde, Crowdy and Roach, 1952) and brown rot of apples and plums (*Sclerotinia fructigena* and *S. laxa*) (Byrde, 1952).

EXPERIMENTAL.

Fallen leaves showing abundant leaf spot infections were gathered in November, 1953, and kept out-of-doors in trays placed on a rack 2 ft. 6 in. above ground level. Each tray was 18 in. square and 4 in. deep, having wooden sides and a top and bottom of fine wire netting. Wooden partitions extending the full depth of the tray divided it into nine equal compartments. A horizontal screen of sacking was placed below the trays to reduce wind disturbance.

Materials.

Five fungicides were used, each at four concentrations:

(*a*) Sodium salt of dinitro-*ortho*-cresol (containing approximately 50 per cent. DNOC) as 0·8 per cent., 0·4 per cent., 0·2 per cent. and 0·1 per cent. aqueous solutions.

(*b*) Sodium pentachlorophenate (sodium PCP) as 0·8 per cent., 0·4 per cent., 0·2 per cent. and 0·1 per cent. aqueous solutions.

(*c*) Phenyl mercury chloride as 0·4 per cent., 0·2 per cent., 0·1 per cent. and 0·05 per cent. aqueous suspensions. The PMC was ground with equal parts of kaolin to form a wettable powder before being made up into a suspension.
(*d*) Triethyl tin hydroxide as 0·4 per cent., 0·2 per cent., 0·1 per cent. and 0·05 per cent. aqueous solutions.
(*e*) Cetyl pyridinium bromide as 0·4 per cent., 0·2 per cent., 0·1 per cent. and 0·05 per cent. aqueous solutions.

Method.

On 26th January, 1954, all the leaves were removed from the trays and batches of 25 complete leaves immersed for five seconds in the respective fungicidal concentrations. The treated leaves were then returned to the trays in such a way that a complete tray was allotted to the treatments with each of the fungicides. The leaves treated with the four concentrations were placed in the eight peripheral compartments (i.e. two for each concentration) while 30 control leaves were placed in the central compartment.

After allowing two hours for drying, the leaves in the trays were placed outside under cover for two days and then remained fully exposed to the weather until the end of May.

Estimation of results.

The effects of the fungicide treatments on *Pseudopeziza ribis* in the overwintered leaves were assessed by estimating the viability of the fungus as shown by its power to discharge ascospores in the spring. On 19th and 20th May the lids of the trays were removed and, being dry, the leaves were immersed in water for three minutes and then allowed to dry for five minutes. Four of the leaves possessing the most mature fructifications were selected from each batch, and a disc one centimetre in diameter cut from each ; these were inverted over 1·7 per cent. agar in Petri dishes. On 24th May the leaves were wet when removed from the trays and were allowed to dry for some time before six more discs were cut and inverted over agar as before. After 48 hours in the laboratory the density of the ascospore discharge from each disc was assessed. The area of greatest density of spores was selected for each disc pattern and the number of spores visible in the low-power field of the microscope counted. A second assessment made two days later indicated no significant changes in the levels of spore discharge.

Results.

Ascospore discharge was inhibited by sodium DNOC, sodium PCP and PMC at all concentrations ; the inhibitory effect of triethyl tin hydroxide was complete only at the two higher concentrations, while the cetyl pyridinium bromide treatment permitted some ascospore discharge at all the concentrations tested.

The effects on the external appearance of the *Pseudopeziza* lesions were closely similar in the leaves treated with sodium DNOC and sodium PCP. At concentrations of 0·8 per cent. and 0·4 per cent., many lesions were marked by small white discs on the upper surfaces of the leaves, caused by the presence of air below the epidermis, following the death of the fungus in the tissues. The lower surfaces of the remainder

of the lesions were slightly ridged at these concentrations. With decreasing concentration the number of white discs was lower, and the development of fructifications on the lower surfaces more pronounced.

On the leaves treated with PMC the external effects of the fungicide were apparent only at the higher concentrations, those of 0·4 per cent. PMC being equivalent to those of 0·1 per cent. sodium DNOC.

Certain observations on the acervuli of *P. ribis* indicate that these are more resistant to eradicant fungicides than are the apothecia. The leaves treated in January with 0·05 per cent. PMC showed acervuli containing malformed conidia when examined in May. Again, in the leaves treated with triethyl tin hydroxide, there was some development of acervuli at the lowest concentration (0·2 per cent.) which prevented ascospore discharge.

Records of other fungal spores discharged from treated leaves showed that PMC was an effective general fungicide even at 0·1 per cent., whereas sodium DNOC and sodium PCP were much less effective against these other fungi than against *P. ribis*.

DISCUSSION.

While PMC proved as effective in these experiments as sodium DNOC and sodium PCP in inhibiting ascospore discharge from overwintered leaves infected with *Pseudopeziza ribis*, there was some indication that the maturation of the fructifications proceeded further in the leaves treated with PMC. The sodium salts, being readily water-soluble, possibly penetrate the leaf tissues rapidly at the time of application, thus halting the development of the fructifications at an early stage. PMC, on the other hand, is almost insoluble in water and may, on application, become fixed in the outer tissues of the leaf. Results on apple canker lesions suggest that PMC thus held has a long duration of fungitoxicity and continues to inhibit ascospore production in the region of the treated surface (Byrde, Crowdy and Roach, 1952). Such an effect may explain the continued growth of the fungus seen in the black currant leaves treated with PMC, combined with an eventual efficacy in inhibiting ascospore discharge.

A comparison between the laboratory findings with the sodium salt of DNOC in 1954 and the results from the field trial in 1953 reflects the contrast between applying a fungicide to leaves by individual immersion in the laboratory, and by a spray on leaves scattered over the ground in a black currant plantation. Whereas in the laboratory trial complete inhibition of ascospore discharge was given by treatment with the equivalent of 0·05 per cent. DNOC, this compound, used as a ground spray in February at twice this concentration, gave only moderately effective control in the field.

It seems unlikely that a satisfactorily uniform wetting could be achieved by spraying aggregations of leaves on the ground, and the movement of leaves by wind action from control to treated plots and vice versa would further reduce the apparent effectiveness of a fungicide applied to an experimental plot. A better result would possibly be obtained by applying the fungicide to the bushes in the autumn, shortly before general defoliation, provided that this did not induce phytotoxic effects on the buds and shoots. Further work on this aspect of the problem is being planned.

SUMMARY.

Five fungicides were tested in the laboratory for the prevention of maturation of the ascospores of *Pseudopeziza ribis* on overwintered black currant leaves. Aqueous solutions of sodium dinitro-*ortho*-cresylate and sodium pentachlorophenate were effective at 0·1 per cent. Phenyl mercury chloride was effective at 0·05 per cent. and triethyl tin hydroxide at 0·2 per cent. Cetyl pyridinium bromide was of little value as an eradicant fungicide in this trial.

ACKNOWLEDGMENT.

Acknowledgment is made to Miss M. Marlow for her help in this trial.

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(*Received* 6/1/55.)

THE DETERMINATION OF THE IRRADIANCE IN VARIOUS SPECTRAL REGIONS FOR PLANT IRRADIATION PRACTICE

Communication No. 2 of the Committee on Plant Irradiation of the " Nederlandse Stichting voor Verlichtingskunde " (Secretariat: Utrechtseweg 210, Arnhem, The Netherlands)

I. Introduction.

In Communication No. 1 of the Committee on Plant Irradiation (1),* it has been recommended that the irradiance for purposes of practical plant irradiation be characterized with the aid of five (or eight, if ultra violet is included) spectral regions. A biological argumentation has been given for the suggested boundaries of the spectral bands.

Our Committee has been asked by many correspondents how the suggested method can be applied in practice and how the required data can be obtained. The present article deals with an outline of a procedure which may be recommended.

It should be remarked that the proposal made in (I) was intended especially for practical plant growers using artificial light ; for the extension services which have to advise growers what to expect from certain lamps for the desired purposes ; for practical growers who want to know more about these problems ; and for laboratories for fundamental and applied research, requiring information about the growth of plants in artificial light, in order to obtain plant material for botanical, horticultural, agricultural, silvicultural or phytopathological research, in so far as the aim of these studies is not primarily of a photobiological nature.

The Committee is aware that the proposed procedure, consisting of a division of the spectrum into a limited number of spectral bands, each containing a biologically important spectral region, is an approximation. However, for the above mentioned purposes, it is considered a justifiable simplification of the factual situation. In fundamental photobiological research, in measurements of action spectra for biological phenomena, the method suggested in (I) is not suitable. For this purpose, narrower spectral bands (or spectral lines from certain lamps) should be used. It is easy to see that the photobiological effect of, for example, a spectral band between 400 and 500 $m\mu$ depends on the distribution of energy within this band. The energy can be distributed evenly or may be concentrated in a single spectral line. For the determination of action spectra, the effects of narrower wave-length bands with known energies should be compared. This type of research will not be considered here.

In Section II the data required for the proposed specification are summarized. Section III deals with methods for obtaining these data by the co-operation of various institutes.

II. Principle of the measurements.

The irradiance on the working plane in each of the spectral bands proposed in (I) can be computed from the following data :

* Further referred to as (I).

1. the curve of the spectral distribution of energy of the radiation from the light source used ;
2. the total irradiance, in milliwatts per square metre, produced by each type of light source used at the working plane.

The percentages of the total irradiance in each of the spectral bands can be determined graphically from the spectral energy distribution curve.

In (I), p. 182, attention was drawn to the fact that radiation in the long wavelength region of the infra red often is not reproducible. Furthermore, specific effects of radiation with wavelengths longer than 1 micron are so far not known. Therefore, the use of a colour filter, which transmits only radiation with wavelengths shorter than 1 micron, might be recommended. This will increase the accuracy of the measurements in the 2nd to the 8th band. If such a filter is used, it is only necessary to know the spectral energy distribution curve of the light sources and the spectral transmission curve of the filter in the wavelength region below 1 micron.

The irradiance on a flat surface can be measured directly with a measuring instrument, the sensitivity of which is independent of the wavelength of the incident radiation, *e.g.* a bolometer or a thermopile. These instruments, however, are not very suitable for measurements in a greenhouse or in the field because of the required high sensitivity of the measuring device.

It is advisable, therefore, that those who wish to apply the procedure recommended by our Committee should use a measuring instrument which allows of the determination of the irradiance upon the plane of reference by simple means.

In the following outline, it is assumed that plants are irradiated mainly from above and that the vertical extension of the plants is relatively small, so that measurements can be made with a flat light meter, perpendicular to the direction of the radiation at the top of the plants (2).

The simplest measuring device is a photo-voltaic cell with a micro-ammeter. For convenient measurements, the reading of the meter should be a linear function of the incident energy up to full scale deflection. However, the sensitivity of the photocell depends on the wavelength of the incident radiation, so that a given deflection does not correspond to equal amounts of radiant energy when light sources of different spectral energy distribution are compared. In order to avoid errors arising from this fact, a *conversion factor* is needed, with the aid of which scale readings can be converted into absolute units for each kind of light source. The conversion factor can be determined by two different procedures :

(*a*) by comparison of the photocell with an instrument, the sensitivity of which is independent of wavelength ;
(*b*) by computation from the spectral sensitivity curve of the cell and the spectral energy distribution curve of each light source.

Thus, for the application of the procedure recommended in an installation for plant irradiation, the following instruments and data are needed :

(i) an instrument for the measurement of the irradiance on the plane of the plants, for which a photo-voltaic cell combined with a micro-ammeter is simple and suitable ;
(ii) the conversion factor(s) for the light source(s) used, for the conversion of meter readings into milliwatts per square metre ;

(iii) the contribution of each spectral band as a percentage of the total irradiance (preferably in the wavelength region below 1 micron).

The following example will elucidate these requirements. The spectral-energy distribution curve between 200 and 1,000 mμ for a high pressure mercury vapour lamp is shown in Fig. 1. About 17 per cent. of the total radiation emitted by the lamp is of wavelength shorter than 1,000 mμ. The percentage of the radiation in each of the spectral bands is given in Fig. 1. If it is assumed that a photocell-microammeter combination has a conversion factor of a mW/m² per scale division for

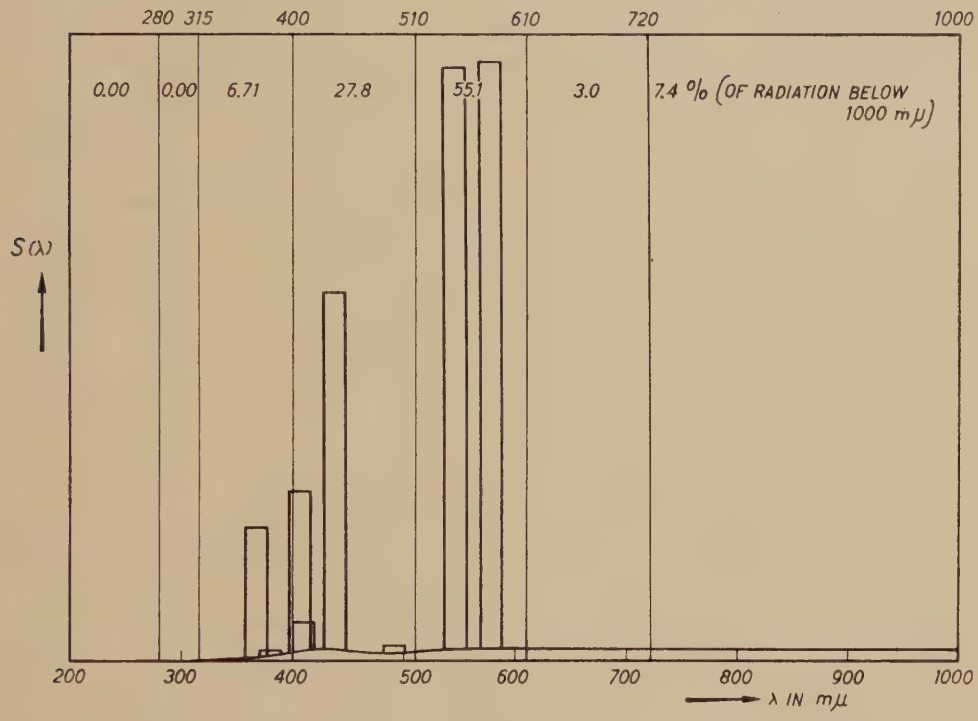

FIG. 1.
Spectral energy distribution curve for a high pressure mercury vapour lamp (1 atm.) between 200 and 1,000 mμ.

the radiation from a high pressure mercury vapour lamp in the wavelength region below 1 micron and that the reading obtained is b scale divisions, then the irradiance in this wavelength region is ab mW/m². From Fig. 1 it can be derived that, for example, the irradiance in the spectral band from 510 to 650 mμ is 0·55 ab mW/m².

Spectral energy distribution curves for an incandescent lamp and one type of fluorescent lamp are given in Figs. 2 and 3 respectively.

In (I), it was proposed that the name " sol " be introduced for the unit of irradiance mW/m². In general, this proposal has not received much support. Furthermore, the name " sol " appeared to be in use already to represent 100 kcal/m²h in daylight measurements (3). *Therefore, it seems appropriate that our committee should withdraw its proposal concerning the introduction of the name " sol ".*

III. Calibration and measuring procedure.

1. Usually, the spectral-energy distribution curve of the light sources in the wavelength region below 1 micron will be supplied by the manufacturer. This curve can also be measured and checked by any laboratory equipped for spectrophotometry. The contribution of the radiation with wavelengths longer than 1 micron can be determined approximately in such a laboratory.

It is conceivable that after some use or by the application of reflectors and other mountings the spectral distribution of energy has changed. The above mentioned laboratories can, if necessary, supply information about these changes.

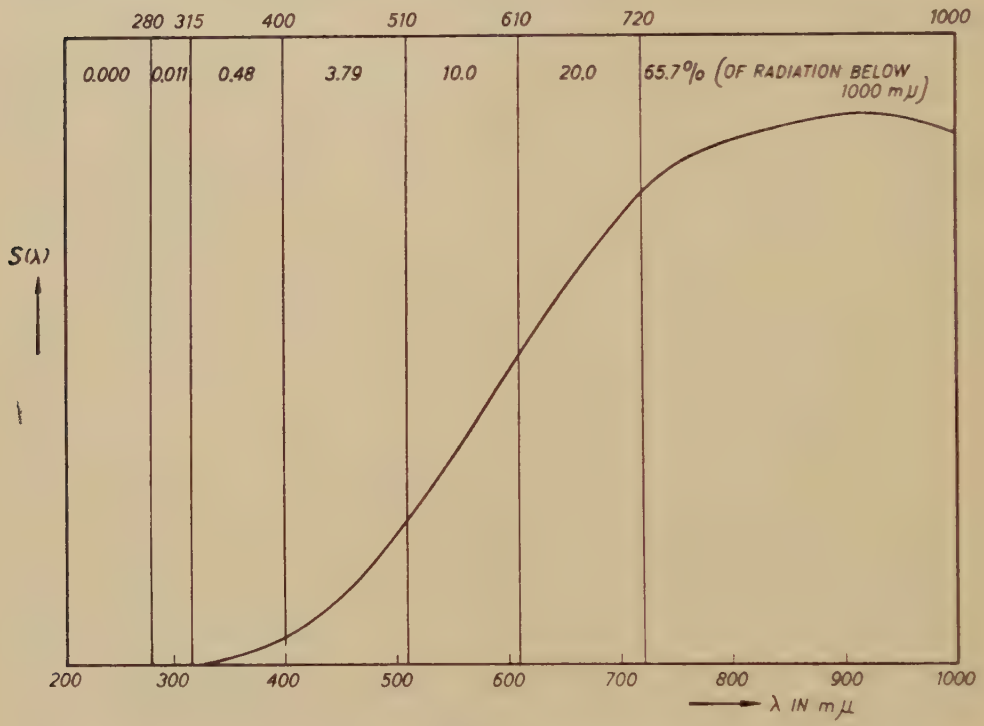

Fig. 2.

Spectral energy distribution curve for an incandescent lamp (200 W.) between 200 and 1,000 mμ.

2. The percentage of the total radiation emitted in each spectral band can be computed from the spectral energy distribution curve of the lamps, either by the user, or by the authority who has measured the spectral energy distribution curve.

3. The irradiance on the reference plane can be measured by the user as a deflection of his measuring instrument. If required (*cf.*, e.g., Section II*b*), the spectral sensitivity curve of the photocell in the instrument can be supplied by the manufacturer, or it can be measured in a laboratory as mentioned under 1.

4. The conversion factor, required to convert the readings of the measuring device into absolute units (mW/m^2), has to be determined for each light source by comparing the reading of the instrument with that of a standardized thermopile or

bolometer in an identical set-up. This can be done by anyone with access to such a standardized instrument, e.g. the user or a laboratory as mentioned under 1. The calibration of an absolute measuring instrument has to be made by especially equipped standardization laboratories. Of course, such a laboratory can also provide the conversion factor(s), but in general this is unnecessary.

In the opinion of our Committee, the necessary data can easily be made available to every user by the co-operation of existing institutions.

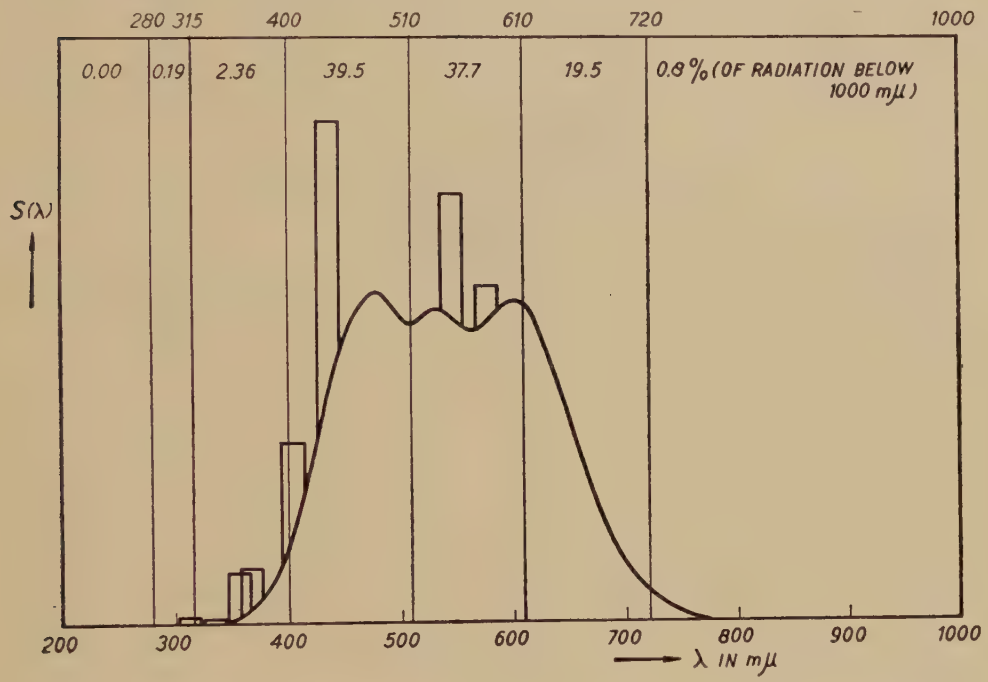

FIG. 3.
Spectral energy distribution curve for a tubular fluorescent lamp (40 W., daylight) between 200 and 1,000 mμ.

IV. ADDITIONAL REMARKS.

When using photo-voltaic cells in a measuring device, some precautions must be taken in order to avoid changes in the calibration in the course of time. Possible causes of these changes are :

1. exposure of the sensitive surface to changing atmospheric conditions, especially to humid air. This can be prevented by an air-tight mounting of the cell.
2. damage to the sensitive surface during exposure to strong light intensities, caused by a too high circuit resistance or by a too large photo-current. This can be avoided by applying a shunt resistance. It is recommended to keep the cell in darkness when not in use.
3. irradiation over longer periods, resulting in fatigue phenomena which seem to be especially noticeable in red light.

The current produced by a photocell is virtually proportional to the incident light intensity. The deviation from linearity increases with increasing circuit resistance (inner resistance of the measuring instrument) and increasing illumination. With a circuit resistance of 100 ohm, these deviations start at currents of 500μA; with a circuit resistance of 1,500 ohm, at currents of 50μA approximately.

It is sometimes stated that the spectral sensitivity curve of a photo-voltaic cell changes somewhat in the course of time; this would result in a change of the conversion factors for the various light sources. A simple check on this can be made by measuring the transmission factor of three coloured glasses with different spectral transmission (e.g. blue, green and red) for the light of a given light source. If this is done for the first time shortly after the calibration of the photocell, it is improbable that the spectral sensitivity curve has changed appreciably as long as these transmission factors remain the same.

As an example of a simple and suitable measuring instrument, Fig. 4 shows an apparatus made for private purposes at the laboratory for Plant Physiological Research of the Agricultural University, Wageningen.

The case F contains a selenium photo-voltaic cell of good quality, mounted air-tight. A convex opaline glass cover over the photocell corrects the dependence on the angle of incidence. The sensitive surface has a diameter of 48 mm. The cell holder is attached to a long hollow brass handle through which the wire connections are drawn. This handle facilitates measurements between plants. The wires are long so that the meter can always be put down in a flat position.

The meter is a microammeter with an internal resistance of 1,500 ohm and a sensitivity of 50μA for full scale deflection. The deviation from linearity is less than 5 per cent. at full scale deflection. Five sensitivity ranges can be chosen with the range switch K that inserts different shunts. The cell produces about 1μA per 10 W/m^2 *visible radiation* (400–720 millimicron*), provided the circuit resistance is low as compared with the internal resistance of the photocell. Mounted as above, however, the photocell produces about 1μA per 40 W/m^2 visible radiation. The measuring range of the apparatus goes up to about 100 W/m^2 (about 30,000 lux). With one neutral filter of about 15 per cent. transmission, full sunlight intensity can be measured.

Generally, any illuminometer of good quality can be used for the measurements described above, provided that the conversion factors have been determined for the light sources used.

It is advisable that the measuring apparatus should be checked from time to time by comparison with an absolute thermopile. If necessary, the user can make this check himself with the aid of an incandescent lamp operated under specified conditions (*cf.* also, Sections III, IV).

SUMMARY.

In order to characterize the radiation in an installation for plant irradiation, the following data are needed:

1. The relative spectral distribution of energy of the light sources in their

* For an incandescent lamp, an irradiance of 1 mW/m^2 in the region from 400 to 720 mμ corresponds to an illumination of 0·20 lux approximately; for a h.p. mercury lamp, to 0·37 lux; for a daylight fluorescent lamp, to 0·27 lux.

PLATE I.

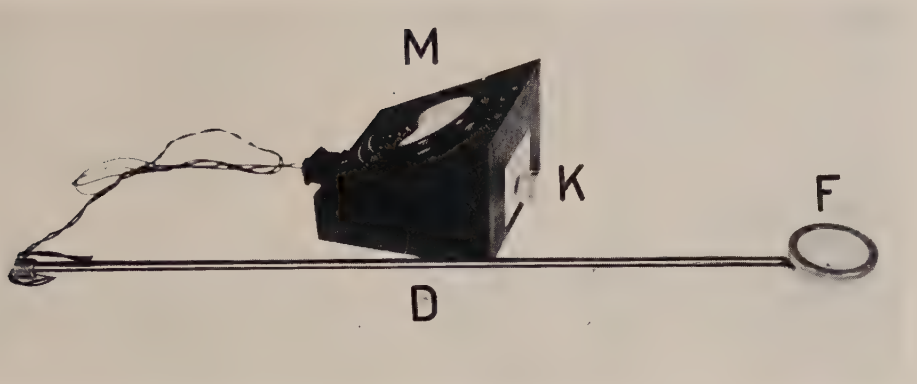

Fig. 4.

Photo-voltaic cell with microammeter. F = photocell, D = measuring box with meter, K = range switch.

[face p. 206

mountings in the wavelength region below 1 micron, to be supplied by the manufacturer or by a spectrophotometric laboratory.

2. The percentage of the total radiation from the light source emitted in the various spectral bands ; these percentages can be computed from the spectral energy distribution curve.

3. The irradiance at the site of the plants (preferably in the wavelength region below 1 micron), to be measured by the user, for example, with a photocell-microammeter combination. This instrument should be calibrated and checked by a laboratory that has access to an absolute bolometer or thermopile. Such a laboratory can provide a conversion factor for each light source ; with the aid of this factor the meter readings may be converted into absolute units (milliwatts per square metre, mW/m^2).

The data mentioned under 2 and 3 enable the user to determine the irradiance in each spectral band. The way in which these data can be obtained is described.

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(*Received* 14/1/55.)

BOOK REVIEWS

MINERAL NUTRITION OF FRUIT CROPS. Ed. by NORMAN F. CHILDERS. (Horticultural Publications, Rutgers University, New Jersey. 1954. Pp. 907, bibl., illus. Price $10.00 or 80s.)

This book, written by 27 American fruit specialists, is an attempt to summarize the work on fruit nutrition over the past 30–40 years. It consists of 18 reviews, 14 of which deal with the nutrition of a wide range of crops, including pome and stone fruits, nuts, citrus, grapes, bush fruits and strawberries.

Each chapter reviews one or more fruit crops and deals with soil, manuring and the essential major and trace elements in relation to plant growth and crop yield, and concludes with an extensive bibliography with complete titles. Although the authors have followed the same general pattern in reviewing their subject, some have enhanced the value of their review by a critical approach based on personal knowledge and experience.

The book includes a large photographic section in which the major and trace element deficiencies and excesses are described and illustrated. The quality and usefulness of the photographs vary considerably. Those of plant organs are on the whole informative but some of the distant views of trees serve no useful purpose. Black and white prints are not the best medium for illustrating certain deficiency symptoms, and this photographic section could be improved by more severe editing and by the inclusion of some good colour plates.

For those readers interested in leaf analysis as a guide to the nutritional status of fruit plants, there is a good review of the method in relation to citrus and a very comprehensive table, with references to author and origin, dealing with the content of nutrients in plant tissues in relation to the incidence of deficiency or toxicity symptoms.

The remaining chapters deal with subjects closely allied to fruit nutrition, namely isotopic tracers, chelated metals and orchard experimentation. The use of isotopes and chelated metals in the study of plant nutrition is relatively new and these two reviews will be of particular interest to the research worker.

The authors of the chapter on experimentation with orchard trees preface their review with the remark that they are " subject matter specialists not biometricians ", but this does not detract from the value of their advice, which is based on 15 years of personal experience in conducting field experiments with trees.

This is essentially a book for the research worker, teacher and fruit specialist rather than for the practical fruit grower. It contains a great deal of useful information and can be recommended as a reference book.

C.B.

FURTHER OUTLOOK. By F. H. LUDLAM and R. S. SCORER. (London : Allan Wingate, Ltd. 1954. Pp. 174, illus. Price 15s.)

This is a book on modern meteorology. It deals mainly with recent advances in knowledge of cloud formations, turbulent air currents, improvements in both the science and the art of weather forecasting, and possible weather control, including rain making and hail prevention. The authors explain how thermal up-currents can

enable not only glider pilots, but also seeds and locust swarms to travel great distances. Experiments in rain making in various parts of the world, by seeding clouds with dry ice, or by providing nuclei of silver iodide smoke, are described and criticized. The authors stress that, although in special circumstances spectacular results may be achieved, the results of any particular operation are still very uncertain. Even if all suitable clouds in an area were seeded, the total annual rainfall would be unlikely to be increased by more than one-tenth. Nevertheless, the authors suggest that, as our knowledge of these processes advances, useful increases in rainfall in certain limited areas may be achieved ; and extension of the same principles may lead to some measure of hail control.

The authors state that although the science of weather forecasting is developing, the final correct interpretation of the data is still an art.

Parts of the book make quite stiff reading for the layman ; but many beautiful cloud photographs help to explain a complex and fascinating story. The book is recommended to everyone interested in the weather.

W.S.R.

THE FRUIT YEAR BOOK. 1955. Pp. 142, illus.
THE LILY YEAR BOOK. 1955. Pp. 155, illus.
THE DAFFODIL AND TULIP YEAR BOOK. 1955. Pp. 140, illus.
THE RHODODENDRON AND CAMELLIA YEAR BOOK. 1955. Pp. 102, illus.
The Royal Horticultural Society. (London. 1954. Price 10s. each.)

The R.H.S. Year Books have become an institution ; that on Daffodils is now making its 20th appearance. Surprisingly, since fruit has so general an appeal, the Fruit Year Book is the junior of the four noted here, being but 8 years old, and, we imagine, owing its birth to the workings of that very energetic cell the Fruit Group, then but recently implanted in the august tissues of the Society. Since then the leaven has worked to some purpose and the Fruit Year Book has annually produced a series of authoritative papers, scientific or practical, which are building up into a valuable digest of modern research and technique in this branch of horticulture. (When the time arrives a 10-year index will be an obvious desideratum, for these volumes are not of the kind to suffer discard.) In this issue stars of some magnitude from the Research Stations at East Malling, Long Ashton, Bayfordbury, Auckland, New Zealand, and from the Society shed light upon recent phases of their work in terms diluted to the comprehension of the common man, and make an interest-holding job of it too. Turning from nature as viewed by Science to nature as seen by Art, the Rev. C. L. Dunkerley presents an illustrated article on some notable early nineteenth-century English fruit books. The superb plates produced in those days with loving care and meticulous attention to detail (even the scab spots were included) have never been surpassed, as anyone who has seen the splendid Pomona Herefordiensis must agree. How an amateur can start a modest fruit garden from scratch and in a few years achieve the Gordon Lennox Cup plus the ability to collect the higher ranking medals whenever he cares to exhibit, is told by Howard Crane in an instructive and inspiring article, refreshingly free from text book banalities. In sum this year fully maintains the reputation of its predecessors.

The Lily Year Book contains accounts of lilies growing in such celebrated gardens as Highdown, Mount Stewart and Windsor, the two former written by their

owners, Colonel F. C. Stern and the Dowager Marchioness of Londonderry. Mrs. Mary G. Henry from U.S.A. describes her very successful attempts to collect and cultivate the lilies of North America, many being on the point of extinction through large scale land development and heavy grazing of their habitats. The article is worth studying, for the information it contains can be known to few and the author has well profited by her determination to observe each species in its native haunts. On the practical side the Year Book contains an account of an inexpensive, labour-saving method of propagating lilies on a large scale in use at Windsor Great Park and a record of experiments at the John Innes Institution, whereby the establishment of a compost sequence for pot grown *Lilium regale* was determined. Lectures and discussions at three meetings of the Lily Group are fully reported. There are besides a number of useful papers which lack of space debars from further particularization.

The Daffodil and Tulip Year Book is somewhat preoccupied with reports of flower shows, competitions, popularity ballots, pedigrees and breeding, probably necessary, but hardly stirring to the emotions of the non-fanatic. However, two brief research reports are included. One is from Rosewarne Horticulture Station at Camborne on the effects of a cold spell on prematurely springing daffodils. It seems to have been drastic for nine different forms of injury are recorded. The other consists of some brief remarks by N. W. Abiss on bud and flower production. For the rest, events in the daffodil world are pretty well covered by contributions from New Zealand (both islands), Tasmania (pink daffodils), Virginia, U.S.A., the Scilly Isles and Ireland. Tulips are also mentioned.

The title of the Rhododendron and Camellia Year Book reflects the sudden return to popular favour of the Camellia, that waxy reminder of buttonholes and graveyards. This may be the swing of the pendulum or just "one of those things no feller can understand". Whatever the cause the urge to get a Camellia into the garden appears at the moment to be universal, and their inclusion for the first time last year in the Rhododendron Year Book resulted in a complete sell out of the publication. Not unnaturally they figure prominently in the current issue. Patrick Synge writes on the Rhododendrons and Camellias at Leonardslee and Colonsay, and Captain N. McEacharn on his large collection of camellias at his Villa Taranto on Lake Maggiore, with special emphasis on their resistance to summer drought and their winter hardiness. The propagation by approach grafting of the 100-year-old *Camellia reticulata* in the temperate house at Kew, less laborious methods having failed, is described and illustrated. The cure of lime induced chlorosis in pot grown rhododendrons by the application of iron sequestrene is of interest. Whether it would be of value to rhododendrons growing outside in an alkaline soil has not been determined.

G.St.C.F.

PLANT CLIMATE AND IRRIGATION. Ed. by SIDNEY A. SEARLE. (Chichester : Chichester Press. 1954. Pp. 155, illus. Price 20s.)

The book comprises a collection of papers by four contributors, S. A. Searle, F. R. Frampton, B. A. Eastwell and P. J. Salter dealing with the control of plant environment, particularly in glasshouses. It is directed to " inform the thoughtful horticulturalist of many aspects of environmental and growth control and help him make correctly the all important managerial decisions on which the success or failure of his business will depend".

The sections by Searle deal with plant climate ; environmental factors in the glasshouse ; the measurement of soil moisture ; calculation of crop water requirement and application to commercial growing. They give a comprehensive, up-to-date review of published work in a clear and readable manner suitable for the grower. No attempt is made, however, to appraise critically the work cited, an omission which detracts from the value of the papers. Most of the recent work on plant growth under controlled conditions has been carried out in America and the results need to be applied with caution to varieties under British climatic conditions. The osmotic theory of growth control put forward in the section on application to commercial growing is an over-simplification of the very complex problem of salt and water relations in the plant and will be challenged by many plant physiologists. On p. 15, it is stated that glasshouse temperature can be lowered in hot weather by running cold water down the glasshouse roof. Trials under English conditions have not confirmed this result and it is uncertain how far the method will be of value in solving this problem here.

Glasshouse heating is dealt with by Frampton in the section on growing in a microclimate. He approaches the problem as a grower and an engineer, and rightly maintains that high velocity hot water in small diameter pipes is the most effective system for maintaining uniform temperature conditions in glasshouses. It is surprising that no mention is made of mixing valves. Coupled with a response regulator they can greatly help the maintainance of uniform temperature conditions as well as effecting economy in heating cost. The writer favours a 2 in. pipe size, but work at the National Institute of Agricultural Engineering suggests that better results can be obtained in most circumstances by the use of $1\frac{1}{4}$ in. pipes.

It is unfortunate that the paper by Salter on the effects of different water regimes on the growth and yield of tomatoes is published as part of a thesis. The format required for a thesis is generally unsuitable for the presentation of such work to the grower. The results are of great value and interest and should have been presented clearly and concisely without the mass of experimental detail and analysis.

The papers should prove useful and stimulating to the progressive glasshouse grower and the book will do a good service if it stimulates work in this country on plant environment and its effect on growth.

G.F.S.

PUBLICATIONS RECEIVED

VARIETIES OF POTATOES. National Institute of Agricultural Botany. Farmers' Leaflet No. 3 (September, 1954).

STRAINS OF SUGAR BEET. National Institute of Agricultural Botany. Farmers' Leaflet No. 5 (revised July, 1954).

STRAINS OF FODDER BEET AND MANGEL. National Institute of Agricultural Botany. Farmers' Leaflet No. 6 (November, 1954).

NOTICE TO CONTRIBUTORS

1. The **Journal of Horticultural Science** is an official organ of the Horticultural and Agricultural Research Station, Long Ashton, Bristol, and the Horticultural Research Station, East Malling, Kent.

The Publication Committee welcome horticultural papers from all other sources.

2. All **manuscripts** should be in double-spaced typewriting, with a good margin, and should be addressed to the Editors, East Malling Research Station, near Maidstone, Kent, England. Papers must be written concisely. Historical reviews should be as short as possible and only such references to literature as bear directly on the subject should be included. Tables and illustrations, if any, must be only such as are absolutely essential for clear presentation.

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4. Each paper must conclude with a concise and critically correct **summary** of its actual content. It should contain any new or verified constants as well as any other critical data of permanent value but not tables or graphs. Any organism mentioned should be given its scientific name.

5. Where a paper is of a highly technical nature it should be preceded by a brief **synopsis**, in which its contents are simply and clearly stated for the benefit of non-technical readers.

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